VOLUME XVIII APRIL, 1935 NUMBER 4

JOURNAL OF DAIRY SCIENCE

PUBLISHED BY THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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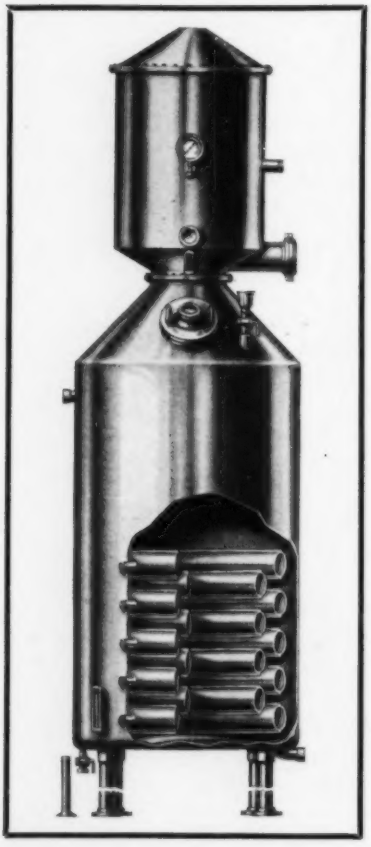

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JOURNAL OF DAIRY SCIENCE

VOLUME XVIII APRIL, 1935 NUMBER 4

A STUDY OF THE INFLUENCE OF THYROXIN ON MILK SECRETION*

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A knowledge of the physiological factors affecting milk secretion is essential to the study of the causes of variation in milk yield among dairy cows. It is reasonable to suppose that the activity of the mammary gland is a direct function of the general metabolism of the animal. If this supposition is correct, those factors that affect the metabolism should have a similar influence on milk yield. The stimulating effect of thyroxin and thryoid extracts upon the metabolic rate of animals has been well established (1, 10, 11). Henderson (9) has studied the effects of various stimulants and galactogogues over two day test periods. McCandlish (13, 14) studied the effect of drug administration on milk yield in a two day period. Ott and Scott (15, 16) injected animal extracts and observed the number of drops of milk secreted in a five minute period following injection. Changes brought about by the various treatments were not significantly large or of long enough duration that the data could be applied to the study of the problem of milk secretion.

Turner (19) has shown that galactin, a hormone elaborated in the anterior pituitary gland, is able to initiate milk secretion in the developed mammary gland. Corner (2) has shown the positive effects of hypophyseal extracts in the hormonal control of lactation. Gowen and Tobey (5) found that injections of insulin and phloridzin caused a marked decline in milk yield. Grimmer and Wentzel (8) claim that thyroidectomised goats secreted milk at a rate comparable to that before thyroidectomy. Graham (6) has studied the effect of thyroidectomy and thyroid feeding on milk secretion and milk fat production of cows. He shows that thyroidectomy and a similar control operation without the thryoidectomy caused a rapid decline in milk secretion and that the effects of the thyroidectomy could not be distinguished from those of the control operation. Thyroid feeding to thyroidectomised cows brought the milk yield level back to that before the operation. Thyroid feeding to normal cows caused a rise in

Received for publication November 18, 1934.

* Publication authorized by the Director of The Pennsylvania Agricultural Experiment Station, November 5, 1934, as Technical Paper No. 662.

milk flow when the rate of lactation was falling but was ineffective when the curve was rising or at its peak.

The study here presented was designed to determine the effect of intravenous thyroxin injections upon the quantity of milk secreted by dairy cows and upon the composition of the milk.

EXPERIMENTAL PROCEDURE

Four cows were injected intravenously with thyroxin. Two purebred Brown Swiss cows, ♯1559 and ♯1562, were used to study the effect of the injections on the composition and quantity of milk secreted. A veterinary physical examination in conjunction with the Brom-thymol-blue test, the cell count, and the chloride content of the milk showed the animals to be free from mastitis which might alter the composition of the milk. ♯1562 was injected with 25 mgm. of thyroxin and after a 10 day interval she was given daily injections of one mgm. for 18 days. Then ♯1559 was given identical treatment for 28 days. ♯1562 was then given a second 28 day period of treatment.

The other cows used were purebred Holstein-Friesians, ♯1525 in declining production and the other, ♯1400, at the peak of her production. They were given 25 mg. injections at seven day intervals for three weeks to study the effect on the quantity of milk secreted.

The following constituents were determined in the milk of the first two cows; Specific gravity with a lactometer, fat percentage by the Babcock method, solids-not-fat calculated by the equation % SNF = L/4 + .2F + .14, lactose by the colorimetric method of Folin and Wu (4) for blood sugar and modified for lactose by Owen and Gregg (17), total nitrogen by the Kjeldahl method and protein calculated as % Protein = N x 6.38, and lactalbumin and lactoglobulin by the colorimetric method of Greenberg (6) for serum proteins. Analyses were made every second or third day on a sample representing an aliquot portion of each milking during the 24 hours.

Body weights were taken on these animals at the beginning and end of each 28 day period. Weighings were made three days in succession at the same hour each day and the three weights averaged to secure the weight for the period.

Data given by Thompson, Thompson, and Dickie (18) show that one tenth of a milligram of Squibb's thyroxin will increase the basal metabolic rate of a person of 140 pounds weight by 10%. On a comparable weight basis one milligram should increase the basal metabolic rate of a 1400 pound cow by 10% and 25 mm. per week should increase the basal metabolic rate about 30%.

EXPERIMENTAL DATA

The accompanying graphs (Figures 1, 2, 3, 4) show the data obtained for daily milk weights, milk fat percentage, pounds of milk fat produced,

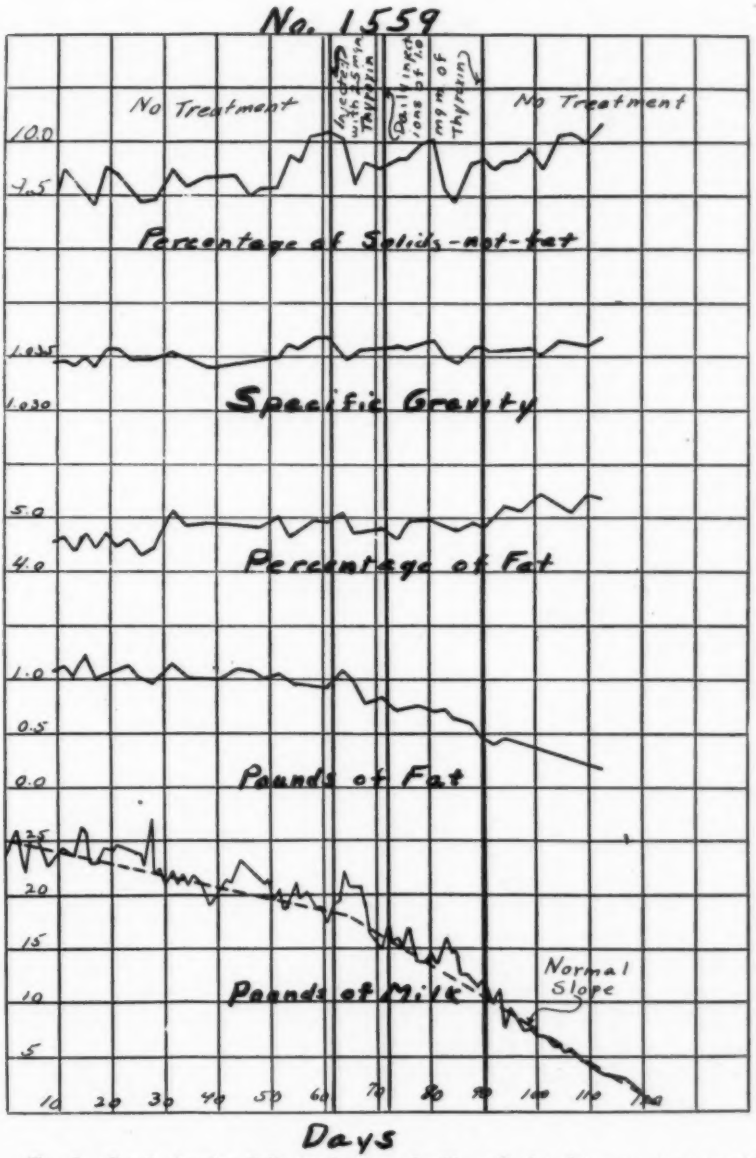

FIG. 1. Graph showing daily production of milk, milk fat, fat percentage, specific gravity, and solids-not-fat for No. 1559.

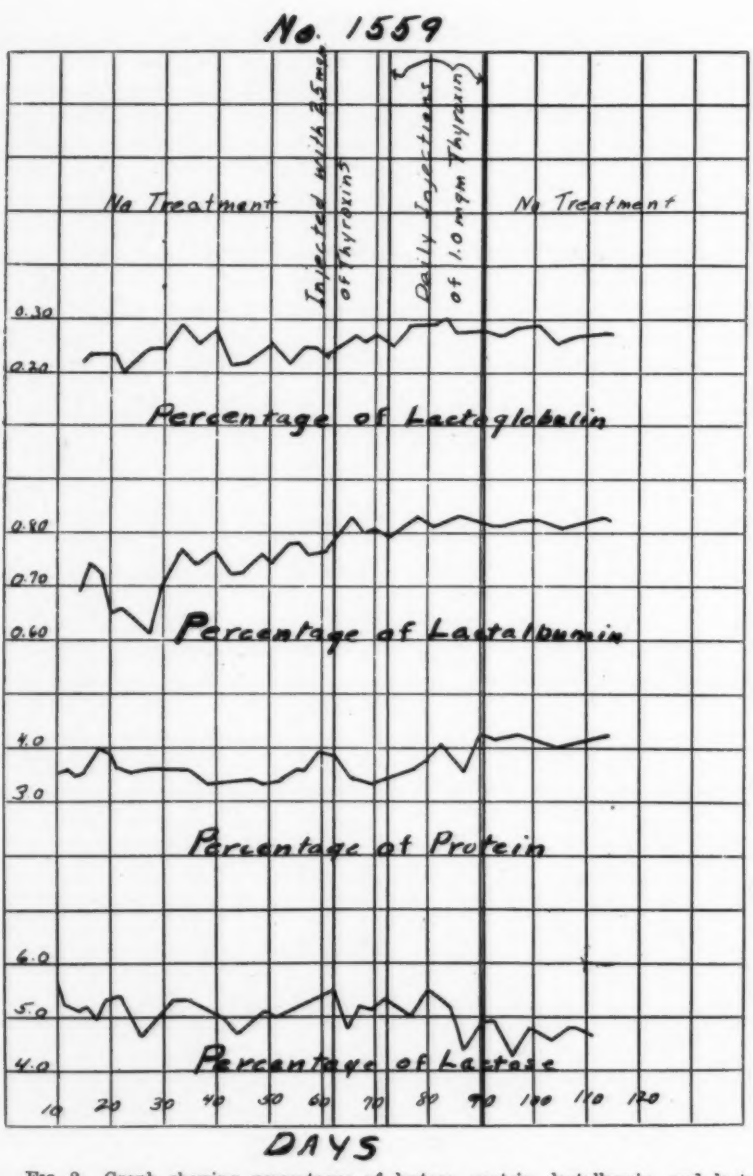

FIG. 2. Graph showing percentages of lactose, protein, lactalbumin, and lactoglobulin for No. 1559.

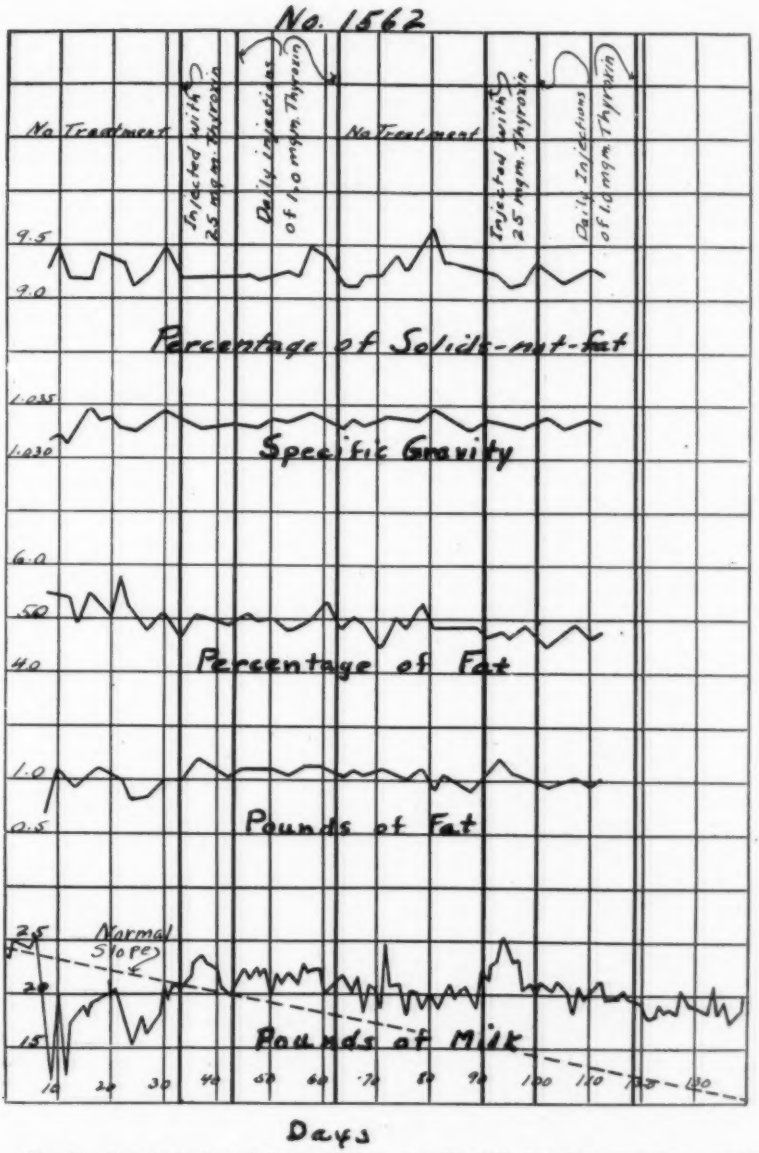

FIG. 3. Graph showing daily production of milk, milk fat, fat percentage, specific gravity, and solids-not-fat for No. 1562.

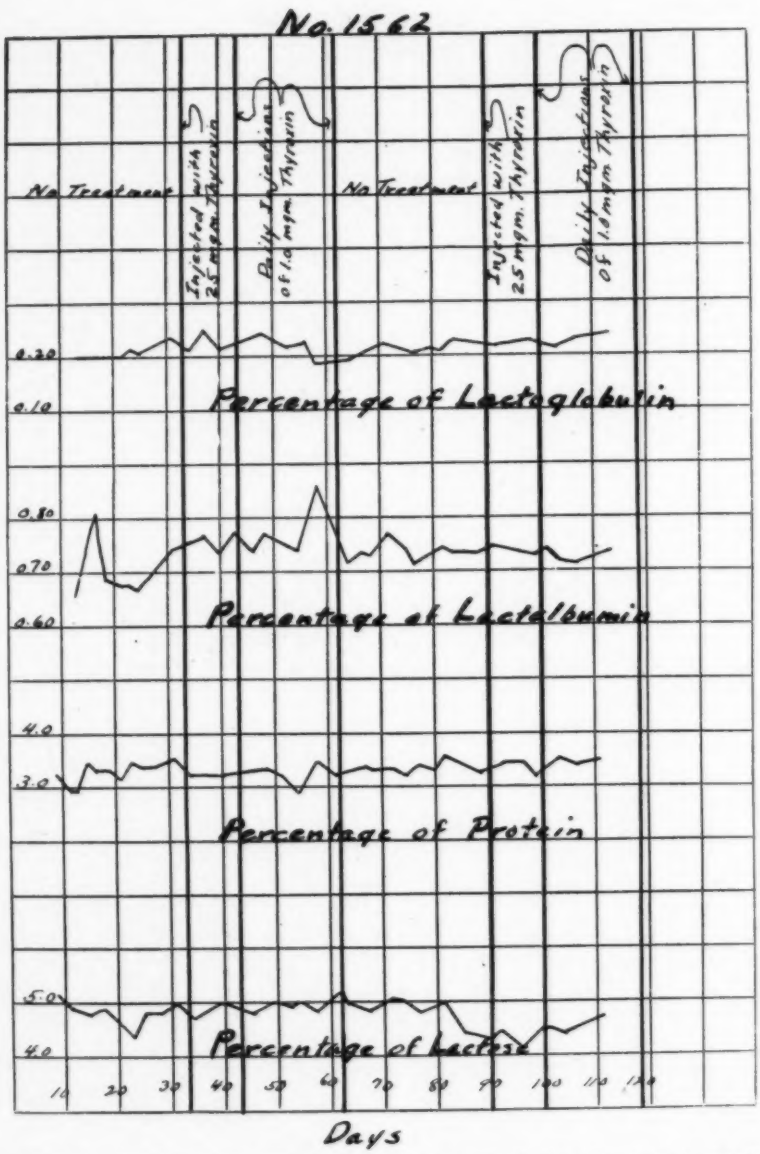

FIG. 4. Graph showing percentages of lactose, protein, lactalbumin lactoglobulin for No. 1562.

lactose percentage, percentage of protein, lactalbumin and lactoglobulin percentages, specific gravity, and percentage of solids-not-fat for cows ♯1559 and ♯1562 used in the first part of the experiment.

The following table shows the average daily production of milk, fat percentage, pounds of fat produced by weekly periods and the body weights of the animals, ♯1562 and ♯1559, by 28 day periods.

No. 1562

Week	*Lbs. Milk*	*% Fat*	*Lbs. Fat*	*Weight*	
12/18	20.4	5.05	1.05		
25	17.7	5.60	0.99		
1/1	18.3	5.25	0.96		
1/8	20.2	4.55	0.93	1215	
15	22.2	5.10	1.14		
22	21.2	4.85	1.03		Injected
29	21.5	5.00	1.07		
2/5	21.8	4.90	1.07	1210	
12	20.7	5.05	1.04		
19	21.0	4.85	1.02		
26	19.4	5.00	0.97		
3/5	19.9	4.80	0.95	1235	
12	23.2	4.75	1.10		
19	20.6	4.65	0.96		Injected
26	20.2	4.80	0.97		
4/2	20.3	4.75	0.97	1265	
		No. 1559			
12/18	23.9	4.55	1.04		
25	23.9	4.60	1.05		
1/1	23.8	4.60	1.03		
8	22.4	4.70	1.01	1175	
15	20.5	4.70	0.96		
22	21.5	4.80	1.03		
29	20.0	4.90	0.98		
2/5	19.2	4.80	0.92	1220	
12	20.1	4.55	0.92		
19	15.7	4.60	0.72		Injected
26	14.3	4.75	0.68		
3/5	13.3	4.50	0.60	1225	
3/12	9.7	4.15	0.40		
19	7.1	4.95	0.35		
26	5.1	4.90	0.25		
4/2	3.1	4.90	0.15	1290	

No. 1559 was dropped to twice daily milking on March 13 and was turned dry early in April because she was due to freshen about June 1. This fact accounts for the rapid decline in her milk yield. All other cows were milked three times daily and were handled in the same manner as the rest of the herd.

The graph (Fig. 5) shows the daily rate of milk secretion of the two

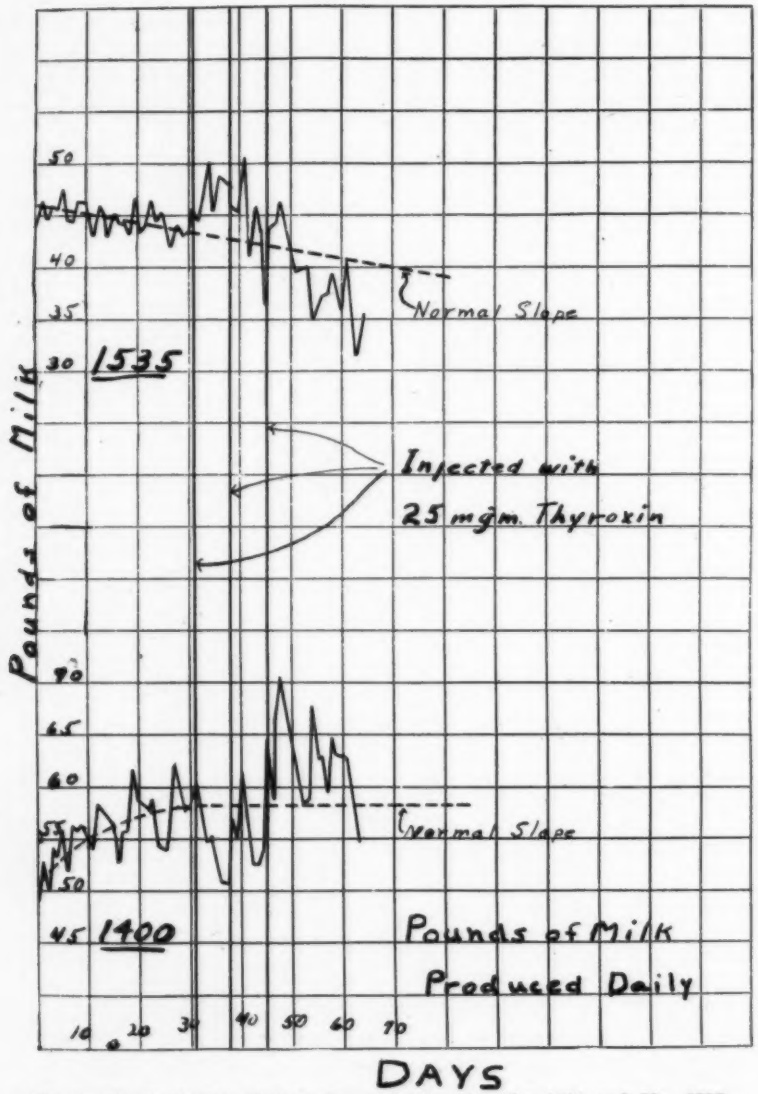

FIG 5. Graph showing daily milk production for No. 1400 and No. 1535.

cows, No. 1535 and No. 1400, used in the second part of the experiment to study the effects on milk yield of weekly injections of 25 mgm. of thyroxin. No. 1400 had been in milk 42 days and apparently was at the peak of her production when the first injection was made. She struggled so violently during the first two injections that a portion was spilled. It is estimated that she received about half of the amount of these injections and the full amount at the third dose. No. 1535 had been in milk six and one half months at the time of the first injection.

DISCUSSION OF RESULTS

The data show that the composition of the milk is not significantly altered by injections of thyroxin. The variations that occur are for the most part no greater than are to be expected, and do not appear to be correlated with the course of the thyroxin injections.

Loeper, LaMaire, and Tonnet (12) have found that in human beings the albumin to globulin ratio in blood plasma normally is 3:2 and that in hyperthyroidism it increases to 9:1, the change being due to an increase in albumin at the expense of the globulin fraction.

It was thought that thyroxin injections possibly would bring about a similar change in these protein fractions in the blood stream, and, provided there is a quantitative exchange of globulin between the blood and the milk in the secretion of this constituent, reflect itself in a lower lactoglobulin value since lactoglobulin is known to be identical with seroglobulin. Such a change did not appear in the milk. Unfortunately blood analyses were not made and so it cannot be said whether or not any change occurred in the blood composition. Such data would have enabled us to speculate with more certainty upon the mode of lactoglobulin secretion.

The most significant change noted was the increase in milk yield in all cases following the administration of thyroxin. In the case of No. 1562 the dosage calculated to be equivalent to a 10% increase in the basal metabolic rate resulted in an increase in milk yield of 11.2%. The increase in yield of No. 1535 resulting from administrations equivalent to a 30% increase in the basal metabolic rate was 6.5%. The two partial injections in No. 1400 caused considerable fluctuation in milk yield with the average somewhat lower than in the two previous weeks. The third complete injection brought about an increase of 9%. The rapid decline in yield of No. 1559 due to the nearness to the end of the lactation period makes a similar computation impossible in her case. With No. 1559 and No. 1562 the 25 mgm. injections were most effective in increasing milk yield and in all four animals so treated the effects were gone at the end of seven to nine days after the injections. The effects of the one milligram injections seemed to disappear as soon as the injections were stopped.

Three different stages in the lactation period are represented by the cows used in this work. The peak of production is represented by No. 1400; she showed a lower milk yield on the two partial doses, but showed an increase following the third dose. This does not substantiate Graham's (6) observations where dessicated thyroid was fed at the peak of production. The period of declining production is represented by No. 1535 and by No. 1562. In both cases the administration of thyroxin was decidedly effective in increasing milk yield. The administration of thyroxin was not particularly effective in increasing milk yield in the case of No. 1559 at the end of the lactation period. This fact indicates that the forces tending to inhibit milk flow at this time are more effective than the stimulating effect of the treatment. In all cases the effects of the administration ceased in a very short time after the injections were discontinued.

Boothby and Sandiford (1) and Thompson, Thompson, and Dickie (18) have found that the effects of thyroxin injections upon the basal metabolic rate can be observed for 30 to 60 days after treatment. The effects upon milk secretion are not so persistent. It would thus appear that the increase in metabolism is not the sole explanation for the increase in milk secretion since the trend of milk secretion does not follow the expected trend of metabolic rate. However, the body weights of the cows indicate that the metabolism is a factor since the animals remained constant in weight or increased very slightly during the periods of injection but gained more rapidly in the periods when they were not injected. This indicates that during the periods of injection the cows were using materials for milk secretion that otherwise would have been used for body tissue.

Turner (19) and Corner (2) have shown that the hormone galactin elaborated in the anterior lobe of the hypophysis has to do with controlling the secretion of the developed mammary gland. Evans and Simpson (3) and Van Horn (20) show that hyperthyroidism results in an increase in the activity of the hypophysis. It would thus appear that thyroxin stimulates milk secretion indirectly by causing an increased secretion of galactin from the anterior lobe of the hypophysis.

SUMMARY AND CONCLUSIONS

Two purebred Brown Swiss cows were injected intravenously with thyroxin alternately over three 28 day periods at a rate calculated to increase the basal metabolic rate by 10 per cent.

The quantity of milk secreted was weighed and analyses of the milk were made to determine the specific gravity, the weight of milk fat produced, and the percentages of milk fat, lactose, protein, solids-not-fat, lactalbumin, and lactoglobulin. Body weights were taken each 28 days.

Two purebred Holstein-Friesian cows were given intravenous injections

of thyroxin at seven day intervals for three weeks at a rate calculated to increase the basal metabolic rate by 30 per cent. Careful observations were made on the quantity of milk secreted by these cows.

An increase in milk secretion resulted from 25 mgm. thyroxin injections. They were most effective during the period of declining lactation just previous to the last few weeks of the lactation period. They were less effective at the peak of production and at the extreme latter end of the lactation period the effect was hardly significant.

The composition of the milk as shown by the constituents for which analyses were made was not significantly altered.

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ACKNOWLEDGEMENTS

Grateful acknowledgement is hereby made to the following for aid in the carrying out of this investigation:

1. E. R. Squibb and Sons for their generosity in supplying the thyroxin used.

2. Dr. J. F. Shigley, Professor of Veterinary Science, for making a preliminary physical examination of the animals.

3. A. O. Shaw and coworkers for data on the udder health of the animals.

4. Dr. A. K. Anderson, Professor of Physiological Chemistry, for advice in planning the experiment.

5. Professor R. A. Dutcher, Head of the Department of Agricultural Biochemistry, for criticism of the plan of the experiment.

6. Professor A. A. Borland, Head of the Department of Dairy Husbandry, for suggestions in the planning of the experiment and without whose approval the work would have been impossible to undertake.

FACTORS AFFECTING ECONOMICAL MANUFACTURE, UNIFORMITY IN COMPOSITION AND QUALITY OF BUTTER

D. H. NELSON

Dairy Industry Division, University of California, Davis

The Bureau of Dairying, United States Department of Agriculture, cooperating with the Dairy Industry Division, University of California, started an extension program in 1922 for the standardization and improvement of butter in California. This program is based on the voluntary cooperation of creameries throughout the State who furnish samples of butter for scoring and other samples taken from the churn to be analyzed by the Kohman Method. The scoring and analyzing is done in the so-called "Butter Standardization Laboratory" where detailed records are kept and duplicate copies are returned to each creamery. The findings of this Laboratory are used as a guide in the standardization and improvement of the butter made in those creameries.

The first report on the progress of this program was published in Bulletin 443 of the California Agricultural Experiment Station by F. H. Abbott who continues to be in charge of the work he began twelve years ago. It is with his permission and assistance that this study is based upon the records of the Butter Standardization Laboratory.

The Laboratory analyzes, by the Kohman Method, from 4000 to 6000 samples of butter each year. These samples are furnished by creameries which manufacture about 60% to 70% of all the butter made in California so that the records reveal a fairly accurate picture of the quality of California butter.

Our present study is concerned with the factors influencing both the economical manufacture and the uniformity in both composition and quality of butter—quality as affected by composition. Such factors include the efficiency and conscientiousness of the employees, the efficiency of the machines used—but probably one of the most important factors is the overrun. The overrun reflects, in a large measure, all of the other factors which affect both economical manufacture and uniformity of product. The butter manufacturer readily learns that other factors being equal, an increase in the overrun reduces the cost of manufacture and increases his profits. Therefore, his problem becomes one of maintaining the maximum overrun. This maximum overrun is restricted by both State and Federal laws which require not less than 80.0% fat in butter. The result of these factors is an attempt by the manufacturer to maintain an overrun which puts on the market a butter containing as close to 80% fat as his facilities will permit.

The records of the Butter Standardization Laboratory show (Table 1) that the average fat content of California Butter is approximately 80.5%.

Received for publication November 9, 1934.

This is considerably lower than the figures usually found in the literature. Five recognized references listed in table 1 give five different "averages" for the fat content of butter, namely, 81.5%, 82.41%, 81.12%, 81.31% and 83.0%. However, Hunziker[1] suggested the possible limitations of his figures by saying "American creameries that standardize the percentage composition of their butter, usually endeavor to secure an overrun that approaches as much as possible the maximum theoretical overrun attainable

TABLE 1

Average percentage composition of butter

SOURCE	NUMBER OF SAMPLES	% FAT	% WATER	% SALT	% CURD
California (1931) (1)	5000	80.60	15.96	2.77	0.67
California (1932) (1)					
Gathered Cream	1500	80.49	16.13	2.70	0.68
Whole Milk	2500	80.53	16.32	2.50	0.65
Hunziker (2)		81.5	15.0	2.5	1.0
Hunziker (2)		80 to 81	15.3 to 15.9	2.5 to 3.5	1.0 to 1.25
Thompson, *et al.* (3)	695	82.41	13.90	2.51	1.18
Fundamentals of Dairy Science (4)	573	81.12	15.46	2.44	0.98
Eckles, Combs and Macy (5)	1000	81.31	15.35	2.31	1.03
Judkins and Smith (6)		83.0	13.9	3.0*	1.1

* Recorded as ash in the reference and no mention made of salt.

(1) Taken from the records of the Butter Standardization Laboratory.

(2) Hunziker, O. F.: The Butter Industry, 2nd Edition, 1927, page 450, 451.

(3) Thompson, S. C., Shaw, R. S., and Norton, R. P.: The Normal Composition of American Creamery Butter. U. S. D. A., B. A. I., Bul. 149 (1912).

(4) Associates of Rogers: Fundamentals of Dairy Science, 1928, page 35.

(5) Eckles, C. H., Combs, W. B., and Macy, H.: Milk and Milk Products (1929), page 201.

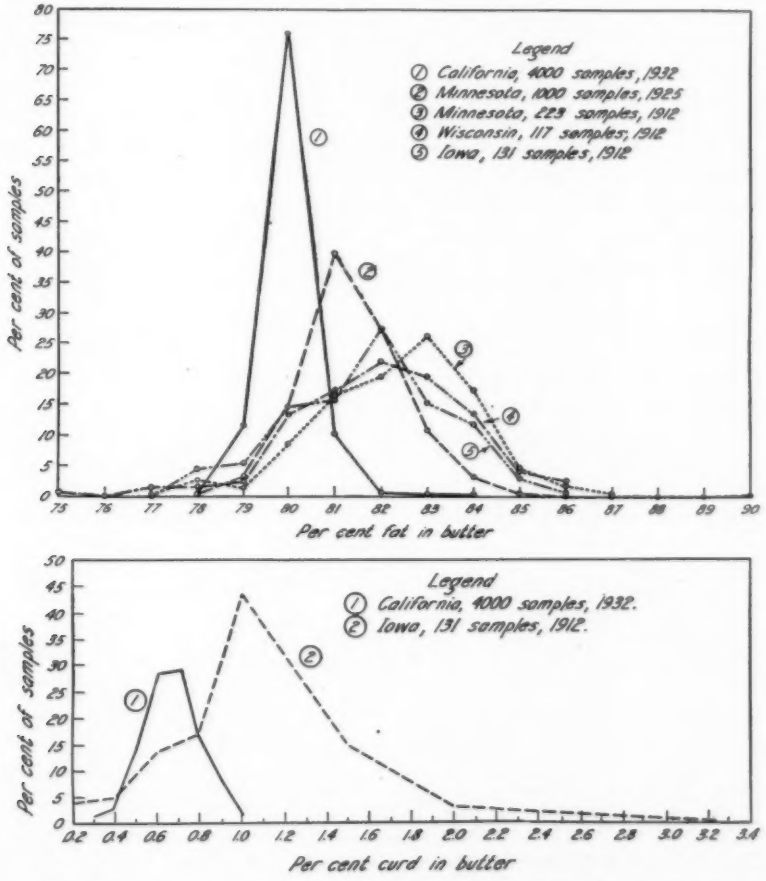
(6) Judkins, H. F., and Smith, R. W.: The Principles of Dairying (1931), page 27.

under the Federal requirement ———." Again in Fundamentals of Dairy Science, page 34, appears "The limits of variations are gradually becoming narrower due to the enforcement of Federal and State standards and to more nearly uniform methods taught by the State dairy school." These facts are demonstrated in figure 1 which shows the percentage distribution, according to fat content of samples analyzed in 1912, 1925, and 1932.

This relationship suggests a statistical treatment with narrower class intervals. The distribution of 4000 samples analyzed in the Butter Standardization Laboratory during 1932 appears in table 2 where the class-intervals give a smooth curve without minimizing the points of special interest, such as the percentage of samples which are only slightly below the State and Federal requirement of 80.0% fat.

[1] Hunziker, O. F.: The Butter Industry, 2nd Edition, 1927, page 451.

About 13% of all the samples appear in those groups containing less than 80% fat and less than 6% in groups containing more than 81.3% fat. Thus the narrow range of 80% to 81% fat is established for the majority of the butter. In other words, the normal percentage of fat in butter is in close agreement with the average percentage given in table 1. Furthermore, the average of the figures given in the literature and quoted in table 1 is calculated as 81.9% fat which falls in the groups including only 6% of the samples tabulated in table 2.


Legend
① California, 4000 samples, 1932
② Minnesota, 1000 samples, 1925
③ Minnesota, 223 samples, 1912
④ Wisconsin, 117 samples, 1912
⑤ Iowa, 131 samples, 1912
Per cent of samples
Per cent fat in butter
Legend
① California, 4000 samples, 1932.
② Iowa, 131 samples, 1912.
Per cent of samples
Per cent curd in butter

This lower fat content of 80.5% as compared with 81.9 means an increased overrun with a consequent increase in profit for the manufacturer. For example, a manufacturer who makes only 300,000 lbs. of butter per year

TABLE 2

Percentage distribution of 4,000 samples according to their fat content

PER CENT FAT	78.00 TO 79.00	79.01 TO 79.74	79.75 TO 79.94	79.95 TO 80.54	80.55 TO 80.84	80.85 TO 81.04	81.05 TO 81.34	81.35 TO 84.00
	%	%	%	%	%	%	%	%
Whole milk	0.6	4.8	6.2	42.3	25.7	10.7	5.1	4.6
Gathered	0.4	8.2	5.5	40.1	19.4	12.1	8.5	5.8
All creameries	0.5	6.0	6.0	41.5	23.4	11.2	6.4	5.0

should have a manufacturing cost of 2.9¢ per lb. according to a survey of 115 Iowa creameries during 1921 and 1922. This 300,000 lbs. of butter having an average fat content of 81.9% would require 245,700 lbs. of fat which would cost $53,562 at 21.8¢ per lb.—the average quotation of the San Francisco market for 1932. The cost of making this butter would be $8,700 and the market value at 21.8¢ would be $65,400. This leaves a profit of $3,138 for the year of 1932. If this same manufacturer purchased the 245,700 lbs. of fat at the same cost of $53,562, but controlled the fat content at 80.5% he would make 305,217 lbs. of butter having a market value at 21.8¢ per lb. of $66,537. Assuming the same cost of manufacture, this would leave $4,275 profit, an increase of 36% over the profit from butter containing the higher per cent fat.

While the fat content of the butter is the important factor in determining the overrun, the buttermaker at the churn usually controls it by a moisture test. This point of view does not give due credit to the salt content and curd content of the butter—both of them being as important in the control of overrun as in the moisture content. It will be seen from table 1 that the average salt content of butter is quite uniformly established at 2.5%.

TABLE 3

Percentage distribution of 4,000 samples according to their curd content

RANGE OF PER CENT CURD	.20 TO .34	.35 TO .44	.45 TO .54	.55 TO .64	.65 TO .74	.75 TO .84	.85 TO .94	.95 TO 1.04
	%	%	%	%	%	%	%	%
Whole milk	1.3	2.6	14.4	29.4	28.3	15.1	7.0	1.9
Gathered	0.9	1.6	10.1	25.7	29.9	19.7	9.3	2.8
Total samples	1.1	2.2	12.8	28.1	28.9	16.8	7.9	2.2

However the curd content is commonly given as 1%—especially when speaking of what Hunziker calls the "curd by difference," which includes all of the solids-not-fat in the butter. This is not in agreement with the figures for California butter as shown by table 1. Again table 3 shows the distribution of 4000 samples received during 1932 in class intervals of their

curd content. The majority of the samples contain between 0.55% and 0.84% "curd by difference," with the average "curd by difference" content at 0.67%. It is significant that none of the samples contained more than 1.0% "curd by difference" and only 10.1% of the samples contained more than 0.85% "curd by difference."

Therefore, from a study of these records, it appears not only desirable but also practical for the butter manufacturer to so control his overrun that the fat content of the butter will always fall between 80% and 81%. In order to do this, it is necessary to use not only the moisture test, but preferably the Kohman Method for analyzing each churning of butter.

THE RELATION OF VITAMIN D TO CALCIUM AND PHOSPHORUS RETENTION IN CATTLE AS SHOWN BY BALANCE TRIALS

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The fact that a calf has a definite vitamin D requirement and suffers from a deficiency of this factor in its ration has been demonstrated by the published work of Rupel, Bohstedt and Hart (1), Bechdel, Landsburg and Hill (2), Huffman (3), and by unpublished work of Gullickson (4) in which experimental "rickets" developed when vitamin D was withheld and disappeared when this factor was added to the ration. Rupel, Bohstedt and Hart (1), and Bechdel, Landsburg and Hill (2) have reported an increased ash percentage in certain representative bones, and an improvement in the concentration of calcium and inorganic phosphorus in the blood plasma following vitamin D therapy of animals suffering from experimental "rickets," which gives indirect evidence of a beneficial effect of vitamin D on the calcium and phosphorus retention, but studies to directly measure this relationship have not been made.

EXPERIMENTAL METHODS

Ten-day mineral balance trials were employed for directly measuring the calcium and phosphorus retention. The mineral retention of normal calves was obtained first to be used for comparative purposes. Young, growing calves were then placed on basal experimental rations relatively low in calcium and phosphorus content, and deficient in vitamin D. Prairie hay was used as roughage for one group, and beet pulp for the other. The concentrates included corn, corn gluten meal, oats, corn starch, and a little wheat bran. For some animals the basal rations were supplemented with calcium, as calcium carbonate, and/or phosphorus, as monobasic sodium phosphate, and/or vitamin D, as viosterol (250 D), or sunshine. Balance trials were run periodically to follow any changes and to note the effect of the various experimental schedules on the calcium and phosphorus retention of the calves. On all calves that developed a vitamin D deficiency as indicated by a rachitic syndrome and a subnormal concentration of calcium and inorganic phosphorus in the blood plasma,

Received for publication October 3, 1934.

[1] The data presented are taken from the thesis by G. Carroll Wallis in partial fulfillment of the requirements for the degree of Ph.D., University of Minnesota, 1934. The problem was suggested by the late Dr. C. H. Eckles who acted as advisor during the first part of the work. Published with the approval of the Director as Paper 1308, Journal Series, Minnesota Agricultural Experiment Station.

particular attention was paid to obtain a balance trial while the calf was still eating well, but after the deficiency was well established. Vitamin D was then added to the ration, and after the lapse of a reasonable length of time, another trial was run.

Except when the experimental schedule required exposure to sunshine the animals were kept indoors in box stalls. A large indoor pen was available for additional exercise. Feeding was done individually and an accurate record kept of actual consumption. All feeds were analyzed for nutrient and calcium and phosphorus content before they were included in the ration. Weights and measurements of height at withers were taken periodically and the rations adjusted at 30-day intervals to furnish somewhat more than the nutrient requirements for growth given by the Minnesota Standard published by Eckles and Gullickson (5). The total calcium and inorganic phosphorus content of the blood plasma were determined from successive 3-day composite samples at least once every thirty days.

Standard methods were followed in conducting the balance trials. In order to accommodate steers in the metabolism stall special equipment was designed,[2] as shown in plate I. A heavy rubber funnel was made from a

PLATE I

E 201 in metabolism stall showing special equipment designed for collecting urine in balance trials.

[2] Credit is due Mr. M. E. Mattison, Curator of the Biochemistry Division, for assisting in designing and making this device.

large automobile inner tube. The top was clamped between two tape-covered metal rings which were then bolted together and bent somewhat to conform to the contour of the animal's body. The funnel opening was held in place over the opening of the animal's sheath by straps around the body. A small-bore, heavy-walled rubber tubing connected to a stopcock cemented into the lower end of the funnel, served to conduct the urine to the collecting pan. The calcium and phosphorus analyses of the excreta and feedstuffs were carried out by the methods of Morris, Nelson, and Palmer (6).

Vitamin D assays using rats were made on the two lots of prairie hay and the beet pulp used in the calf feeding work. The method followed was that given in U.S.P. X Interim revision for the assay of cod liver oil for vitamin D.

OBSERVATIONS

The calcium and phosphorus retention of normal calves: Three Holstein calves, E–180, E–183, and 436 and one Jersey calf, E–181 were used in this work. The ration included prairie hay and a concentrate mixture composed of the common grains and grain by-products. The calves had been exposed to sunshine during the summer and otherwise cared for in the customary manner. They appeared normal and showed a normal concentration of calcium and inorganic phosphorus in the blood plasma. During the balance trials the animals were exposed to sunshine on favorable days. As shown in table 1, these calves, seven to nine months of age, retained an average of 4.17 grams of calcium and 2.61 grams of phosphorus daily.

Experiments using hay as roughage: As soon as the balance trials under normal conditions had been completed, these calves and E–182, a grade Holstein, were started on their respective experimental schedules. All the animals were given an allowance of prairie hay which represented about the amount they would consume *ad libitum*. E–180, E–181, and E–182 ran parallel on a basal ration adjusted to furnish 30 grams of calcium and 15 grams of phosphorus daily per 1000 pounds of live weight. E–180 was kept indoors, E–181 was allowed sunshine exposure on favorable days, E–182 was kept indoors but given a vitamin D supplement of 2 cc. of viosterol (250 D) daily. E–183 and 436 were paired on a similar basal ration but with the phosphorus intake increased to 30 grams daily per 1000 pounds of live weight. E–183 was kept indoors while 436 received sunshine exposure.

After about three months on the experimental program, a series of balance trials were run to note any possible effect of the various procedures on the retention of calcium and phosphorus although none of the animals were showing manifestations of a vitamin D deficiency. The

TABLE 1

The results of mineral balance trials showing the calcium and phosphorus retention of four normal calves

ANIMAL NO.	AGE AT BEGINNING	WEIGHT	TOTAL SUNSHINE	BLOOD PLASMA		MINERAL BALANCE (10-DAY PERIOD)					
						Ca			P		
				Total Ca	Inorganic P	Inake	Outgo	Balance	Intake	Outgo	Balance
	days	*lbs.*	*hrs.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
E–180	290	479	6	9.91	7.46	108.4	53.8	+54.6	77.8	44.9	+32.9
E–181	288	397	6	10.41	7.32	95.6	64.4	+31.2	69.4	47.9	+21.5
E–183	220	415	2	10.48	7.43	119.1	59.4	+59.7	119.6	91.1	+28.5
436	269	465	2	10.15	7.17	132.5	110.9	+21.6	135.1	113.4	+21.7
Average (10 days)								+41.7			+26.1
Daily average								+ 4.17			+ 2.61

TABLE 2

The results of mineral balance trials showing the calcium and phosphorus retention of calves receiving prairie hay as roughage, with and without mineral and vitamin D supplements

ANIMAL	AGE AT BEGINNING	WEIGHT	SUPPLEMENTS		BLOOD PLASMA		MINERAL BALANCE (10-DAY PERIOD)					
							Ca			P		
			Mineral	Vitamin D	Total Ca	Inorganic P	Intake	Outgo	Balance	Intake	Outgo	Balance
	days	*lbs.*			*mgm. per 100 cc.*	*mgm. per 100 cc.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
E–180	431	670	None	None	10.84	5.85	210.3	133.3	+ 77.0	96.4	57.1	+ 39.3
E–182	394	677	None	Viosterol[1]	10.68	7.09	210.7	140.0	+ 70.7	96.4	53.2	+ 43.2
E–183	318	580	Phosphorus	None	9.51	8.03	179.8	68.6	+ 111.2	174.0	128.6	+ 45.4
436	364	622	Phosphorus	Sunshine[2]	10.46	7.72	190.3	98.9	+ 91.4	182.1	150.2	+ 31.9
E–169	610	871	None	Viosterol	9.98	8.13	113.5	48.2	+ 65.3	113.3	78.0	+ 35.3
E–169	680	935	None	None[3]	9.61	7.30	132.7	57.9	+ 74.8	117.8	79.9	+ 37.9
E–170	592	728	Calcium	None	9.45	5.85	573.6	498.7	+ 74.9	91.0	66.3	+ 24.7
E–170	662	787	Calcium	Viosterol[4]	10.62	4.98	664.7	626.8	+ 37.9	96.5	72.5	+ 24.0
Average (10 days)									+ 75.4			+ 35.2
Daily average									+ 7.54			+ 3.52

[1] Two cc. viosterol daily during entire experiment.

[2] Sunshine exposure during entire experiment.

[3] Viosterol removed 17 days previous to this trial.

[4] Five cc. viosterol daily added 17 days previous to this trial.

results of these trials given in table 2 show essentially normal retentions of calcium and phosphorus for all the calves. The viosterol supplement of E–182 had not increased her mineral retention above that of E–180, neither had the sunshine exposure of 436 made possible larger retentions for this aniaml than for E–183. Likewise the increased phosphorus intake of E–183 and 436 had not brought about an increased retention of this element as compared with that of E–180 and E–182. As all of these animals continued through the winter in essentially normal condition no further balance trials were conducted on this group.

The results obtained with E–169 and E–170, grade Holstein females, are also given in table 2. These animals were available from another experiment in which they had developed a rachitic syndrome on a low-calcium-low-phosphorus ration under indoor conditions. E–169 had been growing vigorously throughout the summer after receiving a viosterol supplement with no further change in her experimental schedule. E–170 had shown slow but persistent improvement after the addition of $CaCO_3$ to her ration. Both animals were receiving prairie hay. The results from the first balance trial with each animal indicate that the viosterol was enabling E–169 to retain adequate amounts of calcium and phosphorus from her limited intake, and that E–170 was retaining adequate amounts by virtue of the increased calcium intake without additional vitamin D. The viosterol was now removed from the ration of E–169 and added to that of E–170 at the rate of 5 cc. daily. After seventeen days, balance trials were again run. E–169 was still retaining approximately the same amount of calcium and phosphorus. The added viosterol had not improved the mineral retention of E–170, in fact, it was somewhat less but still within the limits of variations reported for normal animals.

The results of the balance trials run on this group of calves receiving hay as roughage are essentially normal in all cases. None of the calves showed the characteristic symptoms of a vitamin D deficiency. As E–180 and E–183 were on the basal schedule without additional vitamin D for six to seven months it would seem that a reasonable length of time had elapsed in which to deplete the possible vitamin D stores of a young calf at weaning time, hence it seemed more likely that all the animals were getting this factor from some other source. In view of the fact that several investigators have found a varying, yet appreciable, amount of vitamin D in different kinds of hay, it seemed that the prairie hay was the most likely source of an appreciable amount of this factor in the experimental schedule of the control calves on the basal ration. To check this point with approved methods, the prairie hays and beet pulp used as roughage were subjected to vitamin D assays by use of laboratory animals. Both lots of prairie hay were found to carry appreciable amounts of vitamin D which affords a logical explanation for the essentially normal

condition and normal mineral retentions of the calves on the basal ration and the lack of any appreciable differences in the retentions of those getting varying amounts of vitamin D supplements from different sources.

The vitamin D assays with laboratory animals were designed to be qualitative rather than quantitative. Preliminary tests indicated that rats would readily consume the test diets containing 45 per cent of powdered prairie hay and 25 per cent of beet pulp which corresponded to the levels of these materials in the calf rations. The calcium/phosphorus ratio of the rachitogenic diet was changed but slightly when the test materials were added, the tendency being for a wider ratio.

The results of the line test assays carried out according to the requirements of the U.S.P. X Interim revision of the assay of cod liver oil for vitamin D are shown in table 3.

TABLE 3

The results of vitamin D assays by laboratory animals, showing the food intake and the degree of healing for the various test materials

TEST MATERIAL	AMOUNT IN DIET	NUMBER OF RATS	AVERAGE DAILY FOOD INTAKE			AVERAGE DEGREE OF HEALING
			Rachitogenic period	Assay period		
				Test diet	Latent Rachitogenic diet	
	per cent		*gms.*	*gms.*	*gms.*	
Prairie Hay—Lot 45	45	12	6.6	9.8	8.4	1.46
Prairie Hay—Lot 45	30	4	5.9	8.3	7.1	0.75
Prairie Hay—Lot 45	15	4	5.9	7.8	7.2	0.00
Prairie Hay—Lot 55	45	13	6.0	8.5	8.5	2.38
Beet Pulp—Lot 7	25	5	5.3	6.0	6.7	0.00
Rickets Diet—21 day controls	.....	2	5.9	.....	.....	Wide Metaphyses
Rickets Diet—31 day controls	.....	2	6.3	.....	6.5	Wide Metaphyses

[1] The line tests were assigned values of 0, +, ++, etc., and the numerical value of pluses averaged to give the data in this column.

Experiments using beet pulp as roughage: The animals used in this experiment were E–201, a grade Shorthorn steer about one year old, received from a farm in a vitamin D deficient condition; E–185, a grade Guernsey female thirteen months old; and E–194, a purebred Holstein, eleven months old.

After being received from the farm, E–201 was maintained for about six weeks under indoor conditions on a diet similar to that received on the farm. This included two pounds of prairie hay daily and a grain mixture composed of corn, oats, and corn gluten meal. The calf was then in excel-

lent experimental condition for determining the calcium and phosphorus retention of a growing animal suffering from a vitamin D deficiency. The deficiency was evidenced by his rachitic-like condition and the low concentration of calcium and inorganic phosphorus in the blood plasma as noted in table 4, the first balance trial for E–201. After this trial the ration remained the same except for the addition of 5 cc. of viosterol (250 D) daily. The response to this treatment was prompt and marked. The blood picture was normal within three weeks, the stiffness had largely disappeared, the animal was more alert and active, and the appetite was improving. At the end of four weeks he was placed on a balance trial again to ascertain the nature of the calcium and phosphorus retention on the same mineral level but with the animal rapidly approaching a normal condition following the vitamin D therapy. The improvement in calcium retention from 1.44 grams to 6.82 grams daily was nearly five-fold, while the increase in phosphorus retention from 1.10 grams to 2.68 grams daily was slightly less than three fold.

Shortly after the completion of this second trial observations indicated that the animal was apparently normal, whereupon the viosterol was removed from the ration, the experimental schedule otherwise being continued as before. There was a steady decline in the blood plasma calcium from 10.15 to 6.12 mgm., per 100 cc. and in inorganic phosphorus from 8.26 to 5.05 mgm., during the next three and one-half months, at which time the other rachitic-like symptoms began to appear. These observations indicated that body stores of vitamin D, and/or calcium and phosphorus had been depleted and the animal was again in a vitamin D deficient condition. At this time a third balance trial was run, followed by a fourth about five weeks after the ration had been again supplemented with 5 cc. of viosterol. As noted in table 4, the increase in calcium and phosphorus retention was even more striking than before and serves to indicate the important rôle played by vitamin D in promoting the mineral retention of vitamin D deficient calves.

It is conceivable that the level of mineral intake may influence the effect of vitamin D on mineral retention. E–185 and E–194 developed a severe rachitic-like condition on a ration of beet pulp and grain mixture furnishing a very limited calcium intake. A slight temporary improvement in the blood plasma calcium resulted when a $CaCO_3$ supplement was added to bring the intake of E–185 to 40 grams daily and E–194 to 50 grams daily. By the end of a month it was declining again and the physical condition was very poor. E–185 was showing considerable anorexia, and E–194 was also slightly off feed so that the mineral intake indicated in the balance trials run at this time is somewhat lower than the prescribed amounts previously consumed. The results are shown in table 4 as the first balance trial for each animal, respectively. The inability of vitamin D deficient

TABLE 4

The results of mineral balance trials showing the calcium and phosphorus retention of calves, receiving beet pulp as roughage, with and without mineral and vitamin D supplements

ANIMAL	AGE AT BEGINNING	WEIGHT	SUPPLEMENTS		BLOOD PLASMA		MINERAL BALANCE (10-DAY PERIOD)					
							Ca			P		
			Mineral	Vitamin D	Total Ca	Inorganic P	Intake	Outgo	Balance	Intake	Outgo	Balance
	days	*lbs.*			*mgm. per 100 cc.*	*mgm. per 100 cc.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
E–201	.....	527	None	None	6.42	3.56	71.7	57.2	+ 14.5	83.8	72.8	+11.0
E–201	.....	539	None	Viosterol[1]	10.09	6.87	96.4	28.2	+ 68.2	83.8	57.0	+26.8
E–201	.....	775	None	None[2]	6.12	5.05	85.8	79.7	+ 6.1	122.2	116.5	+ 5.7
E–201	.....	786	None	Viosterol[3]	9.29	7.94	102.9	50.1	+ 52.8	119.8	96.4	+23.4
E–185	475	485	$CaCO_3$	None	8.41	3.13	271.2	292.0	− 20.8	38.5	48.8	−10.3
E–185	538	461	$CaCO_3$	Viosterol[4]	11.15	4.98	240.4	184.0	+ 56.4	38.9	21.5	+17.4
E–194	430	575	$CaCO_3$	None	8.30	3.40	356.2	335.5	+ 20.7	48.8	45.6	+ 3.2
E–194	462	625	$CaCO_3$	Viosterol[5]	11.11	6.41	456.0	333.0	+122.4	72.1	32.3	+39.8

[1] Started feeding 5 cc. of viosterol daily 33 days previous to this trial.
[2] Viosterol feeding discontinued approximately 3½ months previous to this trial.
[3] Started feeding 5 cc. of viosterol daily 40 days previous to this trial.
[4] Started feeding 5 cc. of viosterol daily 53 days previous to this trial.
[5] Started feeding 5 cc. of viosterol daily 23 days previous to this trial.

calves to make normal mineral retention on rations supplying an abundance of calcium and normal phosphorus is indicated by the fact that E–185 was in negative calcium and phosphorus balance while E–194 was slightly better than in equilibrium.

A second balance trial was run on each animal after the ration had been supplemented for some time with viosterol. Under the influence of the added vitamin D both animals now showed very satisfactory positive balances of both calcium and phosphorus, as may be noted in the second trial for each animal as recorded in table 4.

Although the appetites were now good, the nutrient and mineral intake of E–185 was held down to the same amount that she consumed on the previous trial, but for unavoidable reasons the mineral intake, especially the calcium, was somewhat larger for E–194 in the second trial than in the first. However, the calcium intake of E–194 was liberal, and more than sufficient in both trials so that the variation undoubtedly does not introduce any appreciable difficulty in interpreting the results. The increase in the calcium intake was approximately 100 grams for the 10-day period, while the increase in retention was about 102 grams. The tendency is for the percentage of mineral retention to decrease with an increase in the intake, but even by assuming that the retention of the extra 100 grams of calcium in the second trial would have been at the same rate as in the first trial there would have been an increase of only about 5.81 grams in the second trial without the added vitamin D. The fact that over 100 grams of extra calcium were retained in the second trial indicates clearly the beneficial effect of the added vitamin D.

The results secured with E–185 and E–194 substantiated the observations already made on E–201 and indicate that a liberal calcium intake will not suffice to promote adequate mineral retention in calves suffering from a vitamin D deficiency.

The relation of vitamin D to aphosphorosis: Two grade Holstein females, E–187 and E–190, twelve and ten months of age, respectively, were used in an attempt to study the effect of vitamin D on the mineral retention of calves suffering from aphosphorosis. The basal ration furnished liberal calcium but a restricted amount of phosphorous, and was typical of those upon which aphosphorosis develops except that vitamin D-free beet pulp was substituted for the usual hay roughage and the calves were kept indoors. By the end of two months it began to appear that uncomplicated aphosphorosis could be obtained only in the presence of at least some vitamin D. The blood plasma calcium as well as the inorganic phosphorus began to decline and the physical condition more nearly resembled that of the rachitic syndrome than simple aphosphorosis. There was no evidence of pica. The onset of physical disturbances was rather sudden and severe with E–190 so that she had to be dropped from the experiment. A balance

TABLE 5

The results of mineral balance trials on E-187 showing the calcium and phosphorus retention on the basal ration,[1] basal ration plus phosphorus, and basal ration plus viosterol.

TYPE OF RATION	AGE AT BEGINNING	WEIGHT OF ANIMAL	BLOOD PLASMA		MINERAL BALANCE (10-DAY PERIOD)					
					Ca			P		
			Total Ca	Inorganic P	Intake	Outgo	Balance	Intake	Outgo	Balance
	days	*lbs.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
Basal	478	694	8.66	3.45	294.5	237.2	+57.3	77.1	53.5	+23.6
Basal plus P[2]	501	700	6.12	5.26	103.9	127.6	−23.7	41.1	63.2	−22.1
Basal plus viosterol[3]	574	630	10.73	7.73	270.5	192.2	+78.3	80.7	34.6	+46.1

[1] Basal ration furnished liberal Ca, restricted P, typical of those producing aphosphorosis except that beet pulp was substituted for the customary hay roughage, and animal was kept indoors. Alterations resulted in development of rachitic syndrome rather than uncomplicated aphosphorosis.

[2] $NaH_2PO_4.H_2O$ added to increase P intake to 18 grams daily 7 days previous to this trial. Animal badly off feed during this trial.

[3] Animal received viosterol for 2 months previous to this trial.

trial was run on E–187 although her condition was not that of simple aphosphorosis. The results of this trial on the basal ration are shown in table 5. The mineral retention is about what might be expected for a mildly rachitic animal.

A second trial was run about a week after a supplement of monobasic sodium phosphate had been added to her ration, to study the effect of added phosphorus on the calcium and phosphorus retention of vitamin D deficient animals, and to learn the nature of the mineral retention of such animals receiving a liberal intake of both calcium and phosphorus. The animal became worse rapidly and went off feed so badly that a satisfactory balance trial to indicate the effect of added phosphorus was not obtained. The results of the trial are shown in table 5. The fact that a severe breakdown seemed to be hastened would indicate that the effect undoubtedly was not very favorable.

The phosphorus supplement was now removed from her ration and viosterol added. As the animal was nearing recovery a third balance trial was run with the ration adjusted to duplicate that of the first trial except for the added viosterol. The results given in table 5 show a considerable improvement in both the calcium and phosphorus retention over that obtained when the animal was mildly deficient in vitamin D and thus substantiate the previous observations as to the favorable effect of vitamin D.

DISCUSSION

The favorable effect of vitamin D on the calcium and phosphorus retention of calves suffering from a vitamin D deficiency is strikingly shown by the results obtained with E–201, E–185, and E–194. The vitamin D deficiency of these animals was indicated by subnormal concentrations of calcium and inorganic phosphorus in the blood plasma, stiffness, bending of the knees, swelling of the knee, hock and pastern joints, humping of the back, and often some inanition. As shown in table 6, the average daily

TABLE 6

The calcium and phosphorus retention of calves before and after vitamin D administration

ANIMAL NUMBER	MINERAL BALANCE (TEN-DAY PERIOD)			
	Before feeding viosterol		After feeding viosterol	
	Ca	P	Ca	P
	gms.	*gms.*	*gms.*	*gms.*
E–201	+14.4	+11.0	+ 68.2	+26.8
E–201	+ 6.1	+ 5.7	+ 52.8	+23.4
E–185	−20.8	−10.3	+ 56.4	+17.4
E–194	+20.7	+ 3.2	+122.4	+39.8
Average (10-day period)	+ 5.1	+ 2.4	+ 74.9	+26.8
Daily average	+ 0.51	+ 0.24	+ 7.49	+ 2.68

retention of these calves at an age when bone mineralization should have been progressing rapidly was only 0.51 grams of calcium and 0.24 grams of phosphorus. When viosterol was added to supply vitamin D, and without any other appreciable change in the ration, there was a marked increase in the calcium and phosphorus retention in every case, the daily average now being 7.49 grams of calcium and 2.68 grams of phosphorus. These amounts represent essentially normal retentions. The increase brought about by the vitamin D administration in from three to seven weeks was approximately fourteen-fold for calcium and eleven-fold for phosphorus. Coincident with the marked improvement in the mineral retention there was a prompt return of the calcium and inorganic phosphorus of the blood plasma to normal concentrations, and a corresponding improvement in the physical well-being of the calf. With such decided and consistent results there can be no doubt of the important rôle played by vitamin D in promoting the retention and utilization of calcium and phosphorus by calves under these conditions.

Increasing the mineral content of the ration of vitamin D deficient calves had no such favorable influence on the mineral retention. Increasing the calcium intake of E–185 and E–194 to 40–50 grams daily failed to promote adequate mineral retention, in fact, E–185 was in negative balance. Although the evidence for the effect of added phosphorus in the case of E–187 is not as conclusive, the fact that adding a liberal amount of phosphorus to the ration of this animal suffering from a mild vitamin D deficiency did not prevent, and may even have hastened, a severe breakdown so that a satisfactory balance trial could not be obtained would cast considerable doubt on the possibility of any beneficial effects of the added phosphorus.

Further informtaion on the mineral retention of normal growing calves is also afforded. Data for the mineral retention of four normal calves, seven to ten months of age, have been given in table 1. As the calves in the experimental group receiving prairie hay as roughage completed the experiment in essentially normal condition, and the rations were similar to those commonly fed in practise, it would seem that these results might also be considered as representative of essentially normal retentions. The data for this group have been given in table 2. When the results of the above two groups are averaged together, the grand average of all the balances with essentially normal calves shows an average daily retention of 6.42 grams of calcium and 3.22 grams of phosphorus. These results corroborate the work of Lindsey, Archibald, and Nelson (7), who obtained normal retentions of 7.74 grams of calcium and 3.52 grams of phosphorus daily for a high calcium group and 4.45 grams of calcium and 2.15 grams of phosphorus daily for a low calcium group.

The average normal daily retention of 6.42 grams of calcium and 3.22

grams of phosphorus obtained in this work is almost exactly in a two to one ratio irrespective of the varying amounts in the ration fed. This closely approximates the ratio of these two elements in the bodies of growing and mature cattle. Lindsay, Archibald, and Nelson (7) noted the same relationship in their work with normal animals. It is of interest to note that in this work the slight average daily positive balances of 0.51 grams of calcium and 0.24 grams of phosphorus shown by the rachitic calves are also in an approximately two to one ratio. Also, that the loss of calcium and phosphorus in the case of E–185 showed this ratio. Such observations indicate an ultimate interdependence between the two elements and suggest that a deficiency in one might be responsible for a lack of retention of the other.

The fact that the calves receiving prairie hay as roughage continued throughout the experiment in an apparently normal condition irrespective of whether or not they received any vitamin D supplement, whereas those on a similar or even higher mineral intake but receiving beet pulp soon showed symptoms of a vitamin D deficiency, indicates that the prairie hay undoubtedly carried an adequate amount of the antirachitic factor, while the beet pulp contributed very little, if any. The vitamin D assays with laboratory animals furnished corroborative and conclusive evidence on the above points. When the two lots of prairie hay were fed to rachitic rats definite healing was obtained, although it was more pronounced for one lot than for the other. No signs of healing were evidenced when the beet pulp was fed.

The assays furnish a basis for estimating the amount of vitamin D supplied the calves by the hay contained in their rations. Rough calculations indicate that the average hay allowance supplied the calves with approximately 135 Steenbock units of vitamin D daily, which served to protect the calves from developing the rachitic-like syndrome on the level of mineral intake furnished by these rations. Just how much less may have sufficed to protect the animals can not be determined from these observations. These estimations indicate that the vitamin D requirement of this species is relatively small as compared with the requirement of infants as recently reported by Hess and Lewis (8), while the fact that an increased mineral intake and changes in the calcium/phosphorus ratio failed to protect the calves or initiate appreciable healing suggests the possibility that calves are less independent of vitamin D than the rat.

Some suggestions as to the amount and length of storage of vitamin D by calves are also indicated by the results obtained. The viosterol fed to the calves as a therapeutic agent supplied roughly 15,000 Steenbock units daily, which is over 100 times the amount furnished by the hay. The exact amount which would have proved sufficient is not known but if the vitamin D in the viosterol had been used efficiently and the excess stored, the animals should have accumulated sufficient for protection for a comparatively long

period of time. When the viosterol was removed from the ration of E–201 after the second balance trial the previous stores of vitamin D made during two and one-half months of viosterol feeding were depleted in about three and one-half months, as evidenced by his physical condition and the low concentration of calcium and inorganic phosphorus in the blood plasma. The time interval would undoubtedly have been shortened had the two pounds of prairie hay allowed part of the time been replaced with beet pulp all of the time. These observations suggest several possibilities. For instance, the vitamin D in irradiated ergosterol may not be the most effective for this species so that the excess available for storage was small, or it may be inefficiently absorbed, or if absorbed, it may be reexcreted. It is also possible that the calf has only limited ability for storing vitamin D at best. The observations on these points are very meager and the possibilities mentioned are made merely as suggestions. The results do indicate, however, that a young growing calf may make a storage of vitamin D under favorable circumstances which may be used as a protection against a deficiency of this factor for a varying length of time under adverse conditions.

CONCLUSIONS

The following conclusions may be drawn from the results of this investigation.

1. The calcium and phosphorus retention of vitamin D deficient calves is very markedly improved by the administration of vitamin D. The average calcium retention may be increased fourteen-fold and the phosphorus retention eleven-fold by vitamin D therapy.

2. Increasing the mineral content of the ration of vitamin D deficient calves has no favorable influence on the mineral retention.

3. Based on the limited evidence obtained, the average daily retention of normal calves approximated 6.50 grams of calcium and 3.25 grams of phosphorus.

4. Judging from the results of this experiment, calcium and phosphorus are retained by normal calves in approximately a two to one ratio regardless of variations in the mineral content of the ration. The statement is equally true for the small average daily retentions made by calves suffering from a vitamin D deficiency. These observations indicate an ultimate interrelationship between these two elements and suggest that a shortage of either one in the ration might act as a limiting factor in the retention of the other.

5. Prairie hay may carry appreciable amounts of vitamin D. Beet pulp, on the other hand, probably possesses very little, if any, antirachitic potency.

6. A young growing calf may store vitamin D under favorable conditions to be used as a protection against a deficiency of this factor for a varying length of time under adverse conditions.

7. Uncomplicated aphosphorosis does not develop when all sources of appreciable amounts of vitamin D are eliminated from rations otherwise similar to those which ordinarily bring about this condition.

8. Vitamin D acts to improve the mineral retention of calves suffering from a rachitic-like syndrome within at least three to seven weeks after its administration.

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EFFECT OF HEATING MILK ON THE TIME WHICH THE CURDS REMAIN IN THE ABOMASUM OF CALVES*

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Soon after coagulation of milk in the stomach the whey begins to separate from the curd. Syneresis of the coagulum, assisted by the peristaltic movements of the stomach, caused the curd to become firmer in texture for some time after the clot has formed. The abomasum of the sucking calf is rarely if ever empty of these curds. As the calf matures, however, and is forced to drink milk from a pail sufficient dilution of the milk sometimes occurs to prevent coagulation. In every case where the milk passes into the rumen on its way through the stomach the strength of the clot is diminished by dilution with the ingesta present there.

The normal healthy calf is physiologically adapted to take care of the curd formed regardless of its tenacity. Gastric juice continues to be secreted for a sufficient length of time to dissolve the nutrients from the coagulum needed for maintenance and growth. When digestion is nearly completed the flow of the digestive juices is retarded sufficiently to avoid wastage. But under abnormal conditions such as disease, the body functions are impaired. One of the first symptoms of a depressed condition is a diminution in gastric secretion. Necessarily, the food should be more digestible at such times. Earlier work (3), (5) has shown that soft curd milk is more digestible than hard curd milk. Brenneman (1), Wallen-Lawrence and Koch (6) have found that heating milk makes it more digestible.

These two viewpoints left the question unsettled as to just what relation heating bore to curd tension. If heating did reduce curd tension it was desirable to find out whether this artificial method of changing the toughness of the curd affected its rate of passage through the stomach in the same way as natural soft curd milk. Besides making these general applications we especially wanted to know the reaction of the calf's digestive system to heated milk.

EXPERIMENTAL

Calves with gastric fistulas were used for these trials. The day before each trial was started the rumen of the calf to be used was emptied manually. Muzzles were then put on the calves and all feed withheld for 12 to

Received for publication October 22, 1934.
* Journal Paper No. J–171 of the Iowa Agricultural Experiment Station. Ames, Iowa. Project No. 47.

18 hours preceding the test. At least one hour before the trial the rumen was washed several times with warm water until the washings were clear. The abomasum was palpated with the fingers to make certain that it was also empty. A stomach tube one-quarter inch in diameter was then inserted into the abomasum by way of the gastric fistula through the reticulo-omasal orifice. The external end of the tube was threaded through the plug used to close the fistula and the plug replaced in the fistula opening. This was done to minimize moisture and heat loss from the rumen.

Two liters of warm milk were then fed through the stomach tube which remained in place throughout the entire trial. Periodically, after feeding, milk was withdrawn from the stomach through this tube into a glass syringe for observation in order to determine the coagulation time of the milk. As soon as the milk had started to whey off, a sample of the gastric juice-whey mixture was drawn for analysis. Subsequent samples of juice were drawn at hourly intervals from the time of feeding.

These samples were tested for hydrogen-ion concentration, free acidity, and total acidity. An attempt was also made to test the peptic activity of the juice, but though two methods were tried each of which gave good results with artificial gastric juice, the results were unsatisfactory with the juice from calves. The milk may have modified the gastric juice so as to interfere with the tests. Probably the pepsin of the gastric juice was adsorbed to the casein.

Curd tensions on all milk fed were obtained before and after heat treatment by a modification of the Hill method (5). The principal difference in the curd test used and that described by Hill was that the authors used commercial rennin instead of pepsin and calcium chloride as the coagulant and that a series of tests were made at two-minute intervals after coagulation.

Milk treated in the following ways was tested for curd tension.

1. Untreated.
2. Boiled for 3 minutes on an oil bath.
3. Heated in an autoclave at 242° F. for 15 minutes.
4. Pasteurized in a vat pasteurizer at 142° F. for 30 minutes.
5. Condensed (plain). This milk was heated to 180° F. during the condensing process.

RESULTS

Typical results of heat treatment on milk are shown in table 1. It will be noted that boiling for three minutes on an oil bath lowered the curd tension in sample 1 to about one-fourth that of the original raw state. In sample 2 there is an even greater difference between the curd tensions of raw and boiled milk. In this case the curd of the boiled milk was very flocculent and when poured from the container broke easily into small

TABLE 1

Typical results of the effect of heat on the curd tension of skim milk

COAGULATION TIME** (MINUTES)	CURD TENSION IN GRAMS*							
	SAMPLE 1			SAMPLE 2		SAMPLE 3		SAMPLE 4
	Raw	Boiled	Autoclaved 242° F.	Raw	Boiled	Raw	Pasteurized	Plain Condensed 180° F. (re-diluted)
10	70	19	0	54	3	72	46	8
13	74	22	0	58	6	81	49	16
16	....	20	0	48	4	85	65	13
19	76	22	0	60	6	92	62	9
22	80	16	0	64	0	....	70	15
25	83	18	0	67	2	98	78	12
28	65	....	0	49	..	93	70	17
31	....	....	0	....	..	....	....	13

* Sample 1 was mixed milk from six cows in the College dairy herd. This milk was used in the digestion experiments. Samples 2, 3 and 4 were obtained from different sources.

** Elapsed time between the introduction of the coagulant and the curd tension reading.

pieces. Heating to 242° F. in an autoclave for 15 minutes lowered the curd tension to a point where it could not be measured with curd knives. Pasteurizing in a vat pasteurizer for 30 minutes at a temperature of 142° F. reduced the tension of the curd about 20 per cent.

Plain condensed skim milk which had been heated during the condensing process to 180° F. was tested for curd tension after being rediluted to the specific gravity of raw milk. Heating at this temperature had not reduced the curd to as friable a texture as that of the milk which had been autoclaved at 242° F. The curd tension of the rediluted plain condensed skim milk was less than that of boiled milk in sample 1 and more than that of boiled milk in sample 2. Pasteurizing milk at 142° F. for one-half hour markedly reduced the curd tension of hard curd milk.

According to Hill (5) pre-heating to 114° F. affected the curd tension only slightly. Heating to 197° F. reduced the curd tension to less than one-third of the original curd tension of the milk. When diluted, sterilized evaporated milk had a curd tension only about one-tenth that of the original milk. From Hill's studies it is plainly evident that heat reduced the curd tension, especially when the temperature reaches 180° F. or above.

EFFECT OF HEAT TREATMENT UPON THE TOTAL TIME MILK REMAINS IN THE ABOMASUM OF CALVES DURING DIGESTION

A total of thirty-two samples (two liters each) of raw, pasteurized, boiled and autoclaved milk were used in these trials. The results are shown in figure 1. At first the evacuation time was determined by palpation of

the stomach contents by passing the hand through the fistula opening and then further inserting two fingers into the abomasum. Later with experience it became possible to estimate the length of the digestion period with a fair degree of accuracy by palpating the abomasum through the medial wall of the rumen. However, the curd which remained after the trial was considered complete was removed and its dry weight determined.

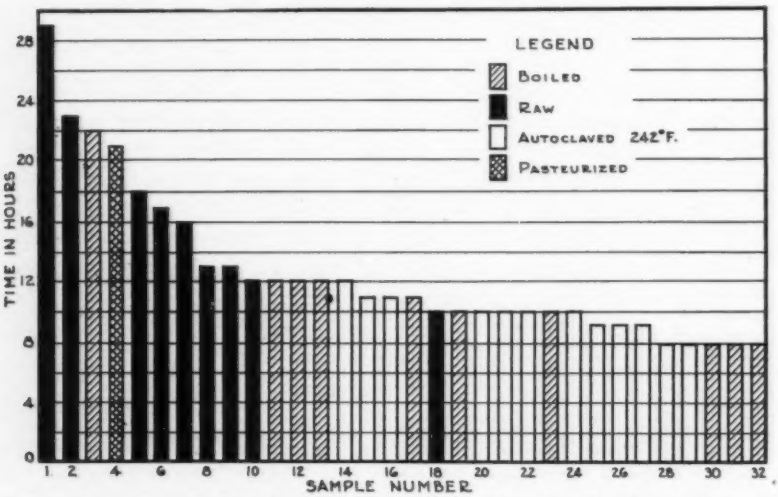

FIG. 1. EVACUATION TIME OF STOMACH WHEN CALVES WERE FED TWO LITERS OF SKIMMILK. END OF DIGESTION DETERMINED BY PALPATION THROUGH FISTULA.

It is evident from figure 1 that a longer time is required for the liquefaction and removal (through the pylorus) of raw milk than boiled or autoclaved milk. Only one trial on pasteurized milk was carried to completion. This test very closely resembled those on raw milk. Boiling and autoclaving milk seem to affect its rate of evacuation from the stomach in much the same way. Both of these methods of heat treatment shortened the evacuation time from that required by either raw or pasteurized milk. The results of the 12 trials carried out with autoclaved milk are much alike, the emptying time of the stomach varying in length from eight hours to twelve hours. The trials with boiled and raw milk were rather uniform except in one case with boiled and two with raw milk which show the influence of the health of the calf on gastric response. These calves were apparently normal as judged by external appearances at the time of feeding. Most digestive disturbances, however, were followed by bowel disturbances, a few hours later.

The emptying time of the stomach usually varied with boiled milk from eight to twelve hours while raw milk required between twelve and eighteen hours. This was longer than was required in another series (4) in which only one liter of milk was used.

In the course of these studies with fistula-calves it was discovered that the curd could be removed from the abomasum without "upsetting" the calf. When the curd was tough, large masses could frequently be withdrawn through the reticulo-omasal orifice without being torn apart. At times curd weighing as much as 75 grams was removed in one piece in this manner. Efforts were then made to trace the digestion of raw and boiled milk by removing the remainder of the test meal after varying periods of time. The weights of the dry curd are listed in table 2.

TABLE 2

Dry weights of curds removed from the abomasum of two calves at varying intervals after feeding two liters of skim milk

	WEIGHTS OF CURD IN GRAMS*					
	RAW MILK				BOILED MILK	
	3rd Hour	6th Hour	9th Hour	16th Hour	3rd Hour	6th Hour
	22.3	23.8	10.9	9.7	36.7	23.4**
	30.2	30.1	10.9		38.4**	25.3
	36.6		15.8		40.9	26.0
	38.0		24.9		43.9**	27.8**
	51.3**				53.9**	
	61.9**					
	65.1**					
Average	43.6	26.9	15.6	9.7	42.6	25.6

* Dried in oven at 75 to 85° C. until weight was constant.

** These samples were siphoned out after crumbling the curd with the fingers and pouring warm water into the abomasum. Siphoned samples are heavier as mucous and residual gastric juice are included.

Although yielding information as to the amount of curd in the stomach at different intervals of time the figures should not be taken as strictly comparable. The trials are too few in number and the curd was not always drawn in the same manner. Because the toughness of the curd increases as the digestion period progresses, it was easier to withdraw a sample which had been in the stomach nine hours than one which had been digesting three hours or even six hours. With proper care six hour samples could usually be removed in one or two pieces, and occasionally curd which had been digesting for only three hours could be removed almost intact. The curd from boiled milk crumbled in pieces if one attempted to withdraw

it after three hours. Occasionally the boiled milk curd broke up when handled after digesting six hours. The samples that crumbled too much were further triturated with the fingers until they were small enough to be siphoned out after pouring warm water into the abomasum. Nine hour weights on boiled milk are not included. Most samples had usually left the stomach at this time.

The limited data indicate that both raw and boiled milk "digest" at much the same rate during the first three to six hours. After this the boiled milk "digests" at a much faster rate. The optimum pH for peptic activity is not reached until about the fifth hour. Perhaps boiled milk is liquefied faster, once the pH of the stomach becomes optimum, because the curd is loose and more friable and permits easier penetration of the digestive fluid.

The method of experimentation used is open to two criticisms: (1) the milk was not mixed with saliva as occurs in sucking or drinking and (2) the fistula may have modified the normal process of digestion. Neither criticism seems especially important to the authors in that the same technique was used in all trials. Besides, according to Dukes (2), the enzymatic action of the saliva of ruminants is relatively unimportant in predigesting proteins, fats and sugars. As to the effect of the fistula on digestion, the

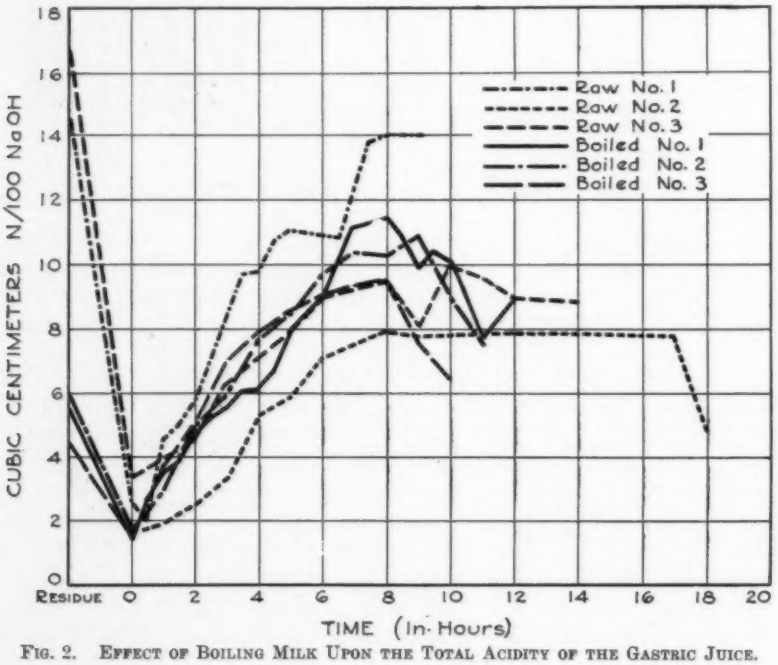

FIG. 2. EFFECT OF BOILING MILK UPON THE TOTAL ACIDITY OF THE GASTRIC JUICE.

acidity curves would indicate that normal physiological processes were occurring in the stomach. Any effect from moisture loss was reduced by replacement of the wooden plug in the fistula during the rial.

EFFECT OF HEAT TREATMENT OF MILK UPON THE FREE ACID, TOTAL ACID AND HYDROGEN-ION CONCENTRATION IN THE STOMACH OF THE CALF

Standard clinical methods were adopted in this experiment with calves. These methods were supplemented (a) by palpation through the fistula, and (b) by removing and weighing the curd at definite intervals after the feeding of the test meal. Part of the analyses during typical trials are shown in figures 2, 3 and 4 because they present interesting information concerning the physiology of digestion in calves.

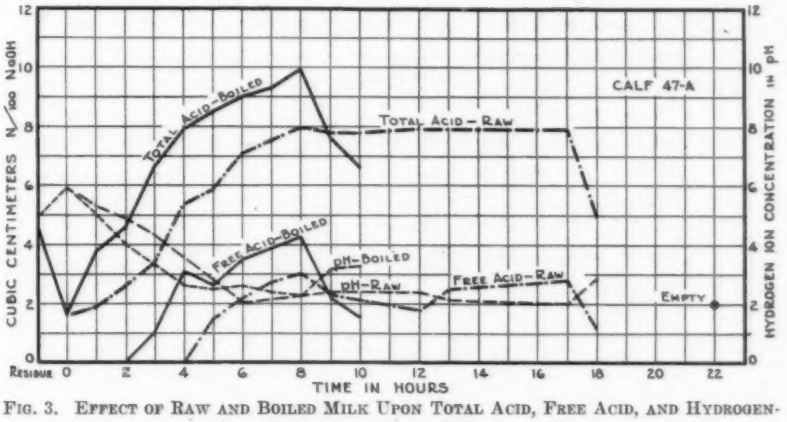

FIG. 3. EFFECT OF RAW AND BOILED MILK UPON TOTAL ACID, FREE ACID, AND HYDROGEN-ION CONCENTRATION OF GASTRIC JUICE AND EVACUATION TIME OF STOMACH.

The results of feeding raw and boiled milk on the total acidity of the gastric juice in calf 47-A are shown in figure 2. It will be noticed that the curves for boiled milk are much the same. The peak in total acid is reached near the eighth hour. From then on the acidity drops rapidly until digestion is complete. Occasionally there are sharp rises as shown in curve No. 1 with boiled milk and curve No. 1 for raw milk. These fluctuations are due to some physiological stimulation beyond the control of the investigator. The curves on raw milk, although different, follow the curves for boiled milk until the eighth hour when the curves for raw milk tend to flatten out rather than drop abruptly.

Data on free acid and hydrogen-ion concentration, as well as total acidity, are shown in figure 3. Again the form of the curves is much the same for raw and boiled milk except that the latter curves are steeper.

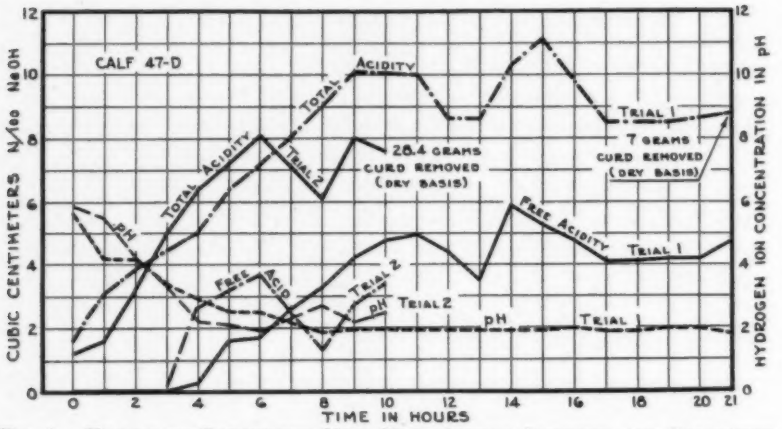

FIG. 4. EFFECTS OF PASTEURIZED MILK UPON GASTRIC SECRETION AND EVACUATION TIME OF STOMACH.

These two tests (Fig. 3) were run on the same calf, 47-A, and were about six days apart.

The results of two tests on pasteurized milk fed to calf 47-A are shown in figure 4. One test was discontinued at the end of 10 hours and the curd removed and weighed. The other test was terminated in 21 hours. A few grams of curd still remained. The tests are too few to be conclusive. However, they indicate that pasteurized milk leaves the stomach in about the same time as raw milk. The acidity curves are much the same. Irregularities in the curves may be due to regurgitation of juices from the duodenum.

From the data which has been presented, and other incomplete trials, it seems that the acidity of the gastric juice is fairly uniform regardless of the processing of the milk. Of course the acidity curves vary but this is likely due to the individuality of the calf. Boiled milk and autoclaved milk leave the stomach faster than raw milk, not because the gastric juice varied in acidity when these milks were fed but more likely because the effect of heat lowers the curd tension and thus allows the gastric juice more curd surface to attack due to the presence of smaller curd masses. Raw milk coagulates in the abomasum of the calf in from one to ten minutes. Boiled milk usually requires about 8 minutes to show the first signs of flocculation and about 15 minutes to really whey off. The minimum time required to coagulate boiled milk was 2 minutes. On the other hand one sample of boiled milk and one sample of autoclaved milk resembled a thick cream soup two hours after ingestion.

One of the authors (F.N.M.) ran a series of trials on himself with aliquot

samples of raw and heat treated milk. Three hundred cubic centimeter portions were used for each trial. Results typical of those secured after drinking boiled milk were obtained when autoclaved milk was drunk. Though the emptying time of the stomach is shorter in the case of man than with calves the acidity curves obtained for each species follow the same general trend. The free and total acid seem to increase at about the same rate in all instances. But the more rapid drop in acidity in the case of the boiled milk would indicate that the heated milk is more easily liquefied, resulting in an early diminution in gastric secretion and rise in pH.

SUMMARY

1. Using a modification of the Hill technique it was found that boiling of skim milk for 3 minutes in an open container on an oil bath lowered the curd tension about 80 per cent. Pasteurizing skim milk at 142° F. for 30 minutes in a vat pasteurizer reduced the curd tension about 20 per cent. Autoclaving at 242° F. for 15 minutes reduced the curd tension to zero. Plain condensed skim milk when diluted to the specific gravity of average skim milk gave a curd tension near that of boiled milk.

2. The rate at which these milks leave the stomach was then studied by palpating the curd mass in the abomasum through the wall of the rumen. Two liters of raw skim milk usually left the abomasum in about 12 to 18 hours when fed by stomach tube. The evacuation time of the same quantity of boiled milk was usually 8 to 12 hours. Equivalent amounts of boiled and autoclaved milk remained in the stomach about the same length of time.

3. At varying intervals of time, curd was removed through a gastric fistula from the stomach of calves. The data indicate that equal amounts of raw and boiled milk are liquefied at much the same rate during the first 3 to 6 hours. After this the boiled milk liquefies at a faster rate.

4. Measurements of the total acid, free acid and hydrogen-ion concentration were made upon the gastric contents of calves which had been fed test meals of equivalent quantities of raw and heat treated milk. Boiled milk and autoclaved milk leave the stomach more quickly than raw milk. This is not because the gastric juice varies in acidity when these milks were fed, but more likely because the effect of heat lowers the curd tension and thus permits the curd to break up more easily. The breaking up of the curd furnishes a greater surface for the gastric juice to attack.

5. Raw milk coagulates in the stomach of the calf in from 1 to 10 minutes. Boiled and autoclaved milk coagulate more slowly in the stomach—usually requiring 8 to 15 minutes.

6. The variability of the results obtained with the same type of test meal fed under as nearly identical conditions as possible are probably due to fatigue, individuality and the state of health of the animal.

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THE GERMICIDAL EFFICIENCY OF LYE AND CHLORINE SOLUTIONS FOR THE STERILIZATION OF MILKING MACHINES AND CREAM SEPARATORS[1]

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INTRODUCTION

The wide-spread use of chlorine compounds and the more recent introduction of alkali compounds for the disinfection of dairy equipment present a question as to their relative efficiencies under dairy farm conditions. In this work an attempt has been made to compare the results obtained with lye (sodium hydroxide) and chlorine (sodium hypochlorite) solutions used in the sterilization of milking machines and cream separators.

EXPERIMENTS WITH MILKING MACHINES

Procedure: To insure a high initial bacterial contamination, 3 gallons of skim milk heavily inoculated with *Escherichia coli* were drawn through each of 3 new milking machines. This was followed by 3 gallons of cold, water, after which the teat cups and tubes were left at room temperature for a period of 48 hours before exposure to one of the disinfectant solutions.

The bacteriological condition of the respective milking machines before and after treatment with disinfectant solutions was determined by drawing 3 liters of sterile water through each unit from a sterile, imitation udder made of tin. The plate count per cc. of this water was used as an index to the sanitary condition of the machine.

Solution Rack Method. Sixteen trials were run in which the germicidal efficiencies of lye and chlorine solutions were compared. In each trial 3 solution racks were used, the reservoirs of which contained water, lye solution, and chlorine solution, respectively.

After samples had been taken for the purpose of determining the initial contamination, the teat cups were attached to the solution racks and filled with the respective solutions. At the end of 48 hours the solutions were drained from the teat cups and tubes, and the bacteriological condition of the machines determined.

In 10 of the 16 trials comparisons were made between the germicidal

[1] Contribution No. 157, Department of Bacteriology, and No. 96, Department of Dairy Husbandry.
Received for publication December 24, 1934.

efficiency of 0.5 per cent lye solution and chlorine solution containing approximately 200 parts per million (p.p.m.) available chlorine, and in the 6 remaining trials between 0.3 per cent lye solution and chlorine solution containing 100 p.p.m. available chlorine. The results of these studies are summarized in table 1.

TABLE 1

A comparison of the germicidal efficiency of lye and chlorine solutions when used with milking machines. (Solution rack method)

	DISINFECTANT USED					
	Water		Lye		Chlorine	
Number of trials	10	6	10	6	10	6
Initial concentration[1]	.....	.....	0.510	0.322	198	101
Final concentration	.....	.....	0.484	0.284	1.5	0
Per cent loss in concentration	.....	.....	5.09	11.8	99.2	100
Initial bacterial count[1]	119,862	165,816	363,030	200,233	152,925	90,750
Final bacterial count[2]	768,300	475,000	2,098	506	1,133	726
Per cent increase or decrease in bacterial count	+540	+186.4	−99.42	−99.74	−99.25	−99.19

[1] Concentrations of the lye solutions are expressed as percentage of sodium hydroxide, and of the chlorine solutions as parts per million (p.p.m.) of available chlorine. The values given in the table are averages of the results of all of the replicate experiments.

[2] The initial bacterial count was determined by making plate counts on 3 liters of water which had been passed through one of the contaminated machines before treatment. The final bacterial count is represented by the plate count per cc. of 3 liters of water which had been passed through the machine immediately after treatment with disinfectant solution or with water in the case of the control. The bacterial counts, therefore, merely serve as arbitrary indexes of the sanitary condition of the machine before and after treatment. The values given in the table are averages of the results of all of the replicate experiments.

The germ-killing efficiency of the chlorine solutions containing approximately 100 and 200 p.p.m. available chlorine proved to be nearly as high as that of the lye solutions. Although the available chlorine was almost entirely depleted at the end of 48 hours, over 99 per cent of the bacteria had been destroyed in all cases.

Immersion Method. Three 8-gallon crocks were filled with water, chlorine solution, and lye solution respectively. Except for the fact that the teat cups and tubes were immersed in the respective crocks, the procedure was identical with that in the preceding trials. In a series of 4 experiments it was found that the average germicidal efficiencies of the chlorine and lye solutions were 96.97 and 99.50 per cent, respectively. The bacterial counts of the check machines, which were treated only with water, showed an average increase of 135.9 per cent for the 4 experiments.

At the conclusion of these trials the chlorine solutions, the average

initial concentration of which was 215 p.p.m., were completely depleted of chlorine. The lye solutions, on the other hand, decreased from an average initial concentration of 0.52 to 0.43 per cent sodium hydroxide at the end of the experiments.

The results of 6 subsequent experiments, carried out under dairy farm conditions, clearly demonstrated the loss in concentration of chlorine solutions when the immersion method was used. The chlorine solutions when placed in the crocks gave an average test of 195 p.p.m., and after 24 and 48 hours use only 98 and 38 p.p.m., respectively. A 0.56 per cent lye solution under the same conditions contained 0.44 per cent sodium hydroxide at the end of one week. It is doubtful if the period of utility of chlorine solutions extends beyond 48 hours when the immersion method of sterilizing milking machine parts is used.

Experiments with cream separators

Procedure: Sterilized parts of the 3 separators were assembled and the machines uniformly contaminated with a mixed flora of microorganisms by pouring through 6 gallons of specially prepared skim milk. One of the separators was designated, for the purpose of a single experiment, as the check machine and was rinsed with 3 liters of sterile water. Bacterial plate counts on this water were accepted as a general index of the initial contamination of the separators. The other separators were then rinsed with 3 liters of one of the various concentrations of disinfectant solutions employed. After treatment, each separator, including the check machine, was flushed again with 3 liters of sterile water. The plate counts per cc. on these samples of water were used as relative indexes of the sterilizing action of the disinfecting solution employed, and, in the case of the check machine, of the mechanical washing effect. Although the values given in the tables are averages of all of the replicate experiments, they reflect quite satisfactorily the general trend of the results of individual experiments. The concentrations of the various disinfectant solutions were determined before and after use.

Comparison of lye (0.5 per cent) and chlorine (200 p.p.m.) solutions. It will be observed in table 2 that the lye solutions lost 14.72 per cent of their alkalinity, whereas the values for available chlorine decreased 58.13 per cent. This loss in concentration of available chlorine should not be regarded necessarily as detrimental, providing the solution was not so depleted that it had lost its germicidal power entirely. A chlorine solution for example which shows the same test before and after use apparently has yielded none of its chlorine to the process of disinfection.

The reduction in bacterial count reflects some interesting points. Under the conditions imposed by the experimental procedure about 82 per cent

TABLE 2

Comparison of lye (0.5 per cent) and chlorine (200 p.p.m.) solutions for the sterilization of cream separators

	WATER	LYE, APPROXIMATELY 0.5 PER CENT	CHLORINE, APPROXIMATELY 200 P.P.M.
Number of trials	12	12	12
Initial concentration*	....	0.489	203
Final concentration	....	0.417	85
Per cent loss in concentration	....	14.72	58.13
Initial bacterial count*	3,512,727	3,512,727	3,512,727
Final bacterial count*	644,000	175,283	19
Per cent reduction in bacterial count	81.66	95.01	99.99

* See footnotes table 1.

of the organisms were removed by water irrespective of the bactericidal power of the solution. The percentage of organisms removed mechanically, however, varies between wide extremes. Experiments not included in this paper were specially designed to evaluate this point. It was found that from 2 to 45 per cent of the organisms were removed by rinsing. The significance of the 82 per cent reported in table 2 is subject to question.

The average bacterial count of the machines after they had been treated with 0.5 per cent lye was 175,283, whereas the machines treated with chlorine solutions containing 200 p.p.m. gave an average count of only 19. (The results of the individual experiments, not given in the table, show that the machines treated with chlorine gave counts of 0 in 10 of the 12 trials, whereas the machines treated with lye solution gave bacterial counts ranging from 2700 to 1,700,00 per cc.).

The consistent failure of the 0.5 per cent lye solutions to sterilize the machine (bacterial count = 0 per cc.), and the consistently low bacterial counts obtained when the separators were treated with chlorine suggests that the rapidity of action of a disinfectant should be considered in selecting a sterilizing agent for cream separators. The superiority of the chlorine may be due to a greater rapidity of action than that of the lye solutions.

The effect of various concentrations of chlorine disinfectant solutions. In 7 replicate experiments using 4 separators in each, comparisons were made of the relative germicidal properties of chlorine solutions containing approximately 200, 100, and 50 p.p.m. available chlorine.

It will be observed in table 3 that the chlorine solutions originally testing approximately 200, 100, and 50 p.p.m. lost in concentration 53.96, 58.42, and 82.00 per cent respectively, and further, that the per cent reductions in bacterial counts were 99.99, 99.89, and 97.70. The solutions originally testing 50 p.p.m. available chlorine were depleted during use

TABLE 3

Comparison of chlorine solutions containing approximately 200, 100, and 50 parts per million available chlorine for the sterilization of cream separators

	WATER	CHLORINE		
		approximately 200 p.p.m.	approximately 100 p.p.m.	approximately 50 p.p.m.
Number of trials	7	7	7	7
Initial concentration*	....	202	101	50
Final concentration*	....	93	42	9
Per cent loss in concentration	....	53.96	58.42	82.00
Initial bacterial count*	2,137,142	2,137,142	2,137,142	2,137,142
Final bacterial count*	596,142	9	2,323	49,016
Per cent reduction in bacterial count	72.10	99.99	99.89	97.70

* See footnotes table 1.

to a point of questionable germicidal value (9 p.p.m.). The results of these experiments indicate that rinsing a separator with a chlorine solution testing approximately 200 p.p.m. gives satisfactory results from the standpoint of the destruction of bacteria. The use of solutions originally containing 100 p.p.m. or less available chlorine is likely to lead to unsatisfactory results.

The effect of various concentrations of lye solutions. In 8 replicate experiments comparisons were made of the relative germicidal properties of lye solutions originally testing approximately 1.00, 0.75, 0.50 per cent.

The results showed the percentage loss of the original concentrations of these solutions to be 15.38, 9.33, and 17.31 respectively. The percentages of reduction in the bacterial counts were 99.45, 99.73, and 99.39 respectively. These results confirm the observations of Myers[1] and McCulloch[2] who showed that the concentration of lye solutions (within certain limits) is not an important factor in its germicidal activity.

The effect of the time of action of lye solutions. The results of previous experiments suggested that in pouring a relatively small volume (3 liters) of 0.5 per cent lye solution through a separator, the time of exposure was insufficient for effective sterilization. Obviously, the time of exposure could be increased by using larger volumes of the lye solution.

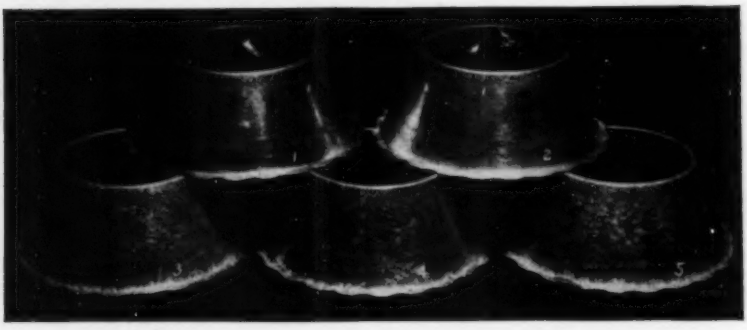
When the times of contact were 0.5, 1.0, 2.0, and 4.0 minutes the aver-

[1] Myers, R. P., 1929. The germicidal properties of alkaline washing powder, with special reference to the influence of hydroxyl-ion concentration, buffer index, and osmotic pressure. Jour Agr. Res., 38: 521–563.

[2] McCulloch, E. C., 1933. The germicidal efficiency of sodium hydroxide. Jour. Bact. 25: 469–493.

age percentages of bacteria destroyed or removed were 98.67, 99.69, 99.96, and 99.99 respectively. The average losses in concentration of the lye solution for the corresponding time intervals were 41.0, 24.8, 12.2, and 7.4 per cent respectively. Although the details of the data are not presented in tabular form, they suggest that when lye solution is used in sufficient volume to require from 2 to 4 minutes to run through the machine, adequate destruction or removal of bacteria may be expected.

Effect of a 0.5 per cent lye solution on separator discs. While it may be possible to bring about adequate destruction of bacteria in a cream separator by the use of a large volume of lye solution, such practice is not to be recommended. It was observed that following the continued use of lye solution for the disinfection of cream separators corrosive action resulted. Accordingly experiments were planned to study the effect of prolonged exposure of separator discs to lye solution.



The corrosive action of 0.5 per cent lye solution on new separator discs.
The periods of exposure were as follows: No.1—control not exposed, No. 2—6 hours, No. 3—10 hours, No. 4—13 hours, No. 5—15 hours.

In each of two experiments, a series of 12 new separator discs were immersed at room temperatures in a 0.5 per cent lye solution. Observations made at frequent intervals up to 15 hours showed appreciable corrosive action after 6 hours' exposure. Virtually all of the tinning on both sets of discs was removed after 15 hours' exposure. The illustration shows one series of discs which had been immersed in a 0.5 per cent lye solution for 6, 10, 13, and 15 hours respectively. Similar results were obtained with the other series of discs.

SUMMARY

Milking machine tubing and teat-cups may be sterilized effectively either with chlorine solutions testing 100 p.p.m. available chlorine, or 0.3

per cent lye solutions, providing the solution rack method is used. When the immersion method is employed, the chlorine solutions are less satisfactory than the lye solutions.

Chlorine solutions proved to be a more effective sterilizing agent than lye when used as a disinfectant rinse for separators. The results indicate that chlorine solutions employed for this purpose should contain at least 200 p.p.m. available chlorine in order to insure effective sterilizing action. Little difference was noted in the bacteriological efficiencies of lye solutions testing 1.00, 0.75, and 0.50 per cent, respectively. Since exposure of separator discs to a 0.5 per cent lye solution tends eventually to corrode the surfaces its use cannot be recommended for sterilizating cream separators.

SEASONAL VARIATIONS IN THE LIPASE CONTENT OF MILK

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In the commercial production of raw cream, there occurs at certain seasons of the year a bitter flavor, accompanied by an odor resembling butyric acid; by an increase in titratable acidity; and by a peculiar, burning sensation at the back of the tongue when the cream is swallowed. The defect usually develops only after the cream has been held in the raw state for from 24 to 48 hours, although occasionally it develops much sooner. Pasteurization always checks the development of the defect, and it usually does not appear except in those cases where the cream must be held in the raw state for some time prior to pasteurization. Skim milk does not show the defect, even though the cream taken from the skim milk becomes very bitter. Since these facts indicate the possibility of fat hydrolysis, an investigation was undertaken to determine the extent to which lipase is present in mixed milk as received at commercial cream plants in New York State, with special reference to seasonal variations.

LITERATURE REVIEW

Although several reports of investigations bearing on the question of the lipase content of both human and cow's milk appeared in the literature prior to 1909 (see references 1 to 17 inclusive), Maass in that year presented the first really convincing evidence that cow's milk contains a true lipase capable of hydrolyzing milk fat (18). Using formalin as a preservative and sterilized cream as a substrate, he found that the addition of raw milk or cream caused an increase in titratable acidity after incubation at 35–38° C., and that this increase was proportional to the amount of inoculum, a fact which is characteristic of an enzymatic rather than of a bacterial process. He reported that the amount of the enzyme present in milk increases at the end of the lactation period. Unfortunately, this excellent work of Maass seems to have been overlooked by most of the susequent workers.

Rogers and his associates (21, 24) reported some work in 1912 and 1913 which seemed to show the presence of lipase in milk, although their work was weakened by the fact that they used esters other than natural milk fat as a substrate. Palmer (32) showed quite conclusively that milk from cows in advanced lactation contains a true lipase, although he failed to demonstrate it in milk from cows not near the end of lactation. (29, 30, 31).

Although other investigators reported work bearing on the subject

Received for publication January 12, 1935.

(references 19, 20, 22, 23, 25, 26, 27, 28), the first work of real significance after that of Maass was reported by Rice and Markley (33), who in 1922 showed that normal milk *does* contain a very active lipase, which sets fatty acids free from milk fat in the absence of bacterial growth. They used boiled cream as a substrate, preserving it by saturating with sucrose, and titrating with alkali to determine increase in acidity. This is the same method used by Maass in 1909 except for the substitution of sucrose for formalin as a preservative.

Beumer (34) and Virtanen (35) reported failure to demonstrate true lipase in cow's milk, but its presence was confirmed by Rice (38), Koestler (36), Koestler, Roadhouse and Lörtscher (37), Roadhouse and Koestler (39) Nair (40), and Davies (42).

In 1932 Dorner and Widmer (41) noted that homogenization of raw milk causes it to become rancid very rapidly, in from one-fourth to two hours, with a bitter flavor and an increase in acidity. This is in accord with the observation of Beatty (25) and of Frazier and Walsh (52) that lipase acts only on the surface of the fat, so that homogenization, by increasing the fat surface, would enable the action to proceed at a more rapid rate. The findings of Dorner and Widmer have since been confirmed in this country by Halloran and Trout (43), Sharp and de Tomasi (45), Trout (46, 47, 54), Doan (50), Doan and Minster (51), and Ramsey and Tracy (53).

The observation of Maass (1909) that the lipolytic activity in milk increases as the lactation period advances has been confirmed by Palmer (32), Leitch (44), Trout (46, 47), Bailey (48) and Csiszar (49). Csiszar (49) notes that lipolytic activity in milk is much more prevalent in winter than in summer.

EXPERIMENTAL

In this investigation, the method that has been used for studying quantitatively the lipase content of milk has differed from those previously used in one important respect. Instead of adding the milk being studied to sterilized cream, the milk was separated (under strictly commercial conditions); the cream so obtained was standardized to a fat content of 40–42 per cent; and fat hydrolysis in this raw cream was followed by titration of the fatty acids set free, using sodium hydroxide and phenolphthalein. These titrations were recorded as the cubic centimeters of tenth-normal sodium hydroxide required to bring nine cubic centimeters of the cream to the phenolphthalein end-point. If the decimal point in these titrations is moved one place to the left, the result will be equivalent to the common method of expressing titratable acidity as "percentage of lactic acid."

By means of this method of investigation, the substrate in which the lipase acted was raw cream separated from the milk being studied. Such a method of procedure has three distinct advantages:

1. The concentration of lipase in the reaction mixture is the highest that can be obtained, there being no dilution by the addition of sterile cream which contains no lipase. Differences that may exist in the lipase content of the milk are therefore more easily measured.

2. The fat exists in the substrate in a normal condition, unchanged by heat.

3. The conditions under which the activity of the enzyme is studied can be exactly like those under which raw cream is produced commercially, which is a distinct advantage because one of the dairy products in which lipolytic activity is most serious is commercial sweet cream which must be held for some hours or days prior to pasteurization.

Three series of data are available for study. The first of these series consists of reports, by a retail milk distributor in the New York Metropolitan area, of the acidity and flavor of shipments of raw cream produced by a small creamery throughout the fall and winter of 1930–1931. The exact conditions under which the cream was produced are not known to the writers, nor is it known what disposition was made of the cream which was defective. The data are taken from strictly routine commercial reports, none of the people involved in the production or inspection of the cream having any knowledge that any problem of this nature was being studied. The data are available to the writers because our organization had been active as an intermediary in the sale of the cream, and they are included because they show very well the seasonal nature of the difficulty; because they bring out the close correlation between acidity and flavor; and because they offer a very clear-cut substantiation, from a strictly commercial standpoint, of the data included in the other two series of experiments, which were conducted under more carefully controlled but also under somewhat more artificial conditions.

The words used on the inspection reports to describe the defective flavor of the cream were "bitter," "strong," "old" and "poor." The results are given in Table 1.

TABLE 1

Monthly variations in titratable acidity and percentage of poor-flavored shipments

MONTH	CC. OF N/10 NaOH TO TITRATE 9 CC. OF CREAM	PERCENTAGE OF SHIPMENTS REPORTED AS HAVING OFF-FLAVORS
October	1.20	12.7
November	1.29	16.7
December	1.34	58.4
January	1.30	40.2
February	1.27	19.0
March	1.27	6.1
April	1.24	0.0

An examination of these data shows very plainly the close correlation existing between high acidity and poor flavor. The peak for both comes in December, when one would ordinarily expect bacterial activity to be at a minimum.

The second and third experiments were carried out at cream plants where the writers had the privilege of directing procedure. In both cases the cream was separated under commercial conditions at 85°–90° F. from mixed milk from a large number of herds, and was produced to be pasteurized immediately rather than to be shipped raw. The experimental samples were taken from the cream prior to pasteurization.

In Experiment II the procedure was very simple. Each raw cream sample was held in the plant cold-room in a sterile bottle at a temperature near 40° F. for 48 hours and the acidity was then determined as follows: a 9 cc. pipette was filled to the mark with the cream to be tested, and this cream was then allowed to run into a 100 cc. beaker. The cream sticking to the inside of the pipette was rinsed into the beaker with two 9 cc. portions of water, four drops of 0.5 per cent alcoholic phenolphthalein solution were added, and the mixture was titrated to a distinct but faint pink color with tenth-normal sodium hydroxide, and the number of cubic centimeters of alkali were recorded. Table 2 shows the monthly averages of these results.

TABLE 2

Monthly variations in production per day per dairy and in titratable acidity of cream when 48 hours old

MONTH	NUMBER OF SAMPLES	PRODUCTION PER DAY PER DAIRY	CC. N/10 NaOH PER 9 CC. OF CREAM
August	20	167	1.09
September	21	159	1.22
October	21	181	1.19
November	22	167	1.40
December	21	166	1.55
January	22	182	1.65
February	20	198	1.60
March	14	216	1.06
April	9	223	1.05

In the two experiments described above the only means of preventing the activity of microorganisms was by keeping the milk and cream cold. No bacteria counts are available to show how successful this control may have been. The third experiment was planned to give more assurance of the absence of bacterial growth, and bacteria counts were made so as to check on the absence of growth.

In this experiment three 10 cc. aliquot portions of each sample of raw cream (40% fat) were saturated with powdered sucrose to prevent bacterial

growth, the method first used by Rice and Markley (33). The first sample was examined immediately, the second after 48 hours, and the third after 96 hours of digestion at 20° C. (68° F.) Each sample was diluted to 100 cc. with sterile water, 1 cc. was removed for further dilution for bacteria counts, and the remainder titrated to the phenolphthalein end point with N/10 NaOH.

In a few cases a fourth sample was prepared, bacteria counts being made immediately without addition of sucrose. These showed no immediate reduction in the numbers of bacteria due to the addition of sucrose. The sucrose did prevent growth in every case, and usually resulted in a gradual decrease in the number of bacteria.

A few bacteria counts were made on the tributyrin agar described by Anderson (55), to detect the presence of fat-splitting bacteria.

The results are summarized in Table 3, monthly averages only being shown to save space. The bacteria counts shown in Table 3 are the monthly averages of the highest of the six counts obtained for each individual sample.

The data of Table 3 are shown graphically in Figure I, with the production curve drawn inversely to show more clearly any inverse correlation between production and acidity.

TABLE 3

Month by Month variations in production, bacteria counts, and titratable acidity of cream

MONTH	NUMBER OF SAMPLES	PRODUCTION PER DAY PER DAIRY	HIGHEST BACTERIA COUNT	cc. N/10 NaOH PER CC.	
				fresh Cream	Cream digested 96 hours
February 1933	2	208	240,000	0.80	2.30
March	2	208	130,000	0.85	2.20
April	2	213	290,000	0.70	2.00
May	3	233	290,000	0.80	2.00
June	2	230	360,000	0.80	1.65
July	1	Milk Strike	560,000	0.80	1.60
August	...	Milk Strike	.....	.....	.....
September	1	186	430,000	0.80	1.90
October	2	190	230,000	0.70	2.20
November	1	186	400,000	0.90	2.80
December	1	200	120,000	0.90	3.20
January 1934	1	214	190,000	0.90	2.70
February	3	214	200,000	0.90	2.50
March	2	222	192,000	0.90	2.30

There are several significant points to be observed from an examination of Figure I. The variations in the titratable acidity of the cream immediately after skimming are too small to have any significance. Under conditions such as those in this experiment, where lipolytic activity is encouraged

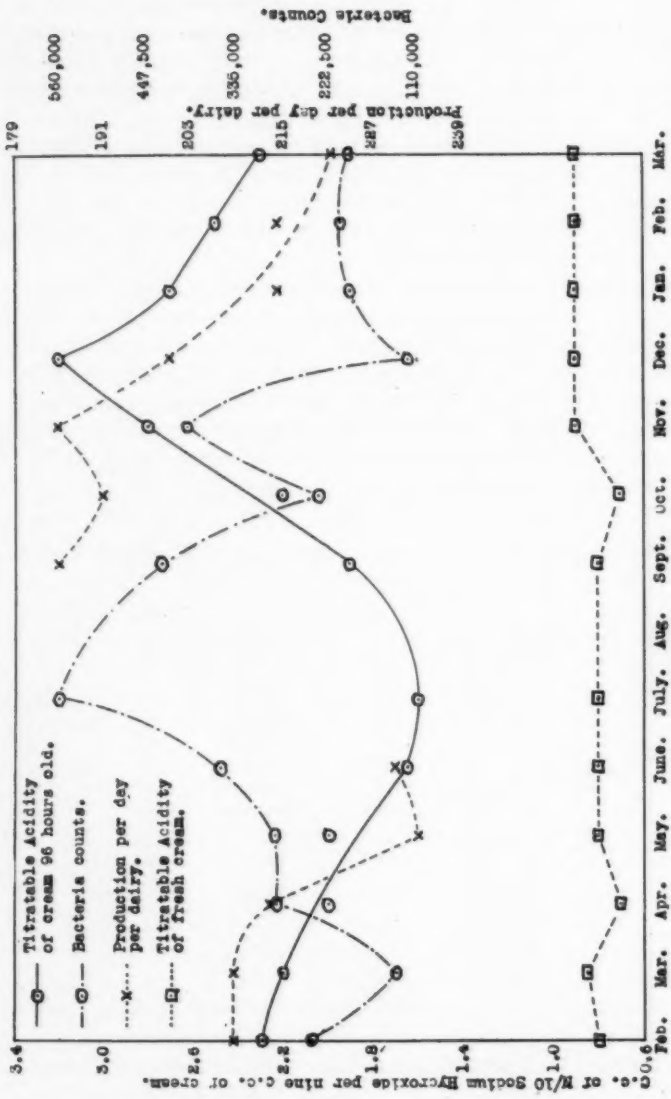

FIGURE I. SEASONAL VARIATIONS IN TITRATABLE ACIDITY, PRODUCTION PER DAY PER DAIRY, AND BACTERIA COUNTS.

and bacterial activity is prevented, some lipolytic activity (as indicated by the increases in acidity in 96 hours) can be demonstrated in the milk throughout the entire year, although this lipolytic activity is at a minimum during early summer (June and July), while it reaches a maximum in early winter (December).

Maass (18) and others have stated that the amount of lipase in milk increases as the lactation period advances. If this be true, then there should be a close inverse correlation between the rapidity of fat hydrolysis and the "production per day per dairy," which is the average weight of milk delivered to the plant by the individual farmer.

An examination of Tables 2 and 3 and Figure I will show that there is a distinct tendency toward such an inverse correlation. (Data on production for July and August are not shown in Table 3 and Figure I because a milk strike among the producers affected the receipts somewhat at this plant during these two months). However, the data of both Tables 2 and 3 show production minima in September without corresponding acidity maxima. This is undoubtedly explained by the fact that these September minima in production are due to a lack of feed (pastures being poor and grain feeding not yet having started) and to the additional fact that low production due to lack of feed is not accompanied by any corresponding increase in the lipase content of the milk.

There is a second production minimum in each set of data, occurring in December in Table 2 and in November in Table 3. There is a corresponding acidity maximum in each case, but it lags one month behind the production minimum. It is believed that the explanation for this lies in the fact that some other factor, in addition to late lactation, is operative in tending to increase the lipase content of the milk, and that the summation of these two lipase-producing factors reaches a maximum one month later than the lactation minimum.

Figure III also shows the monthly averages of the highest bacteria counts obtained on each individual sample. As might be expected, the bacteria counts are somewhat erratic, but there is evident a general tendency for acidity to be low when bacteria counts are high, and vice versa. This is easily understood when we consider that the low acidity occurs in the summer months, and the high acidity in the winter months. In no case is the number of bacteria great enough to account for any increase in acidity, so that the lipase could not have been of bacterial origin.

In 1934 Anderson (55) proposed the use of standard nutrient agar with one per cent of added tributyrin to detect fat-splitting bacteria. A few counts on this agar were made, and the results are shown in Table 4. A glance at this table will show that the numbers of fat-splitting bacteria are far too low to account for the increases in acidity. Thus, in the experiment of March 20, 1934 the acidity increased from 0.90 cc. to 2.40 cc. in 96 hours,

TABLE 4

Titratable acidity and number of both total and fat-splitting bacteria

DATE OF EXPERIMENT	AGE OF CREAM IN HOURS	CC. N/10 NaOH PER 9 CC. OF CREAM	BACTERIA COUNTS ON STANDARD AGAR			FAT-SPLITTING BACTERIA		
			No sucrose incubated 5 days at 20° C.	Sucrose incubated 5 days 20° C.	Sucrose incubated 2 days 37° C.	Sucrose incubated 5 days 20° C.	Sucrose incubated 5 days at 20° C. plus 2 days at 37° C.	Sucrose incubated 2 days at 37° C.
2/13/34	0	0.90	200,000	144,000	400,000	1000	.....	.....
	48	2.10		140,000	120,000	.....	.....	.....
	96	2.50		53,000	70,000	1000	.....	.....
2/20/34	0	0.90		90,000	88,000	.....	.....	.....
	48	2.10		81,000	48,000	.....	.....	.....
	96	2.40		39,000	25,000	1000	.....	.....
3/13/34	0	0.90	152,000	149,000	149,000	1000	5000	4000
	48	2.10	84,000	45,000	45,000	3000	12000	2000
	96	2.20		52,000	42,000	1000	6000	1000
3/20/34	0	0.90	193,000	235,000	180,000	1000	14000	6000
	48	2.10		318,000	112,000	.....	.....	6000
	96	2.40		133,000		2000	15000	5000

while the maximum total bacteria count was 235,000 and the maximum count of fat-splitting bacteria was 15,000.

CONCLUSIONS

1. The increase in acidity, with accompanying bitter flavor, which occurs at certain seasons of the year in raw cream that has been held for some time at ice-box temperatures, is due to the activity of lipase.

2. This lipase is not of bacterial origin, but is secreted by the cows with the milk.

3. The amount of this lipase in milk produced in New York is at a minimum in early summer (June or July), and reaches a maximum in early winter (December or January). There is some lipase present in milk throughout the year.

4. There are two factors which govern the amount of lipase secreted with the milk. One of these is the lactation cycle, the amount of lipase increasing as lactation is prolonged. The nature of the other factor is not known.

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THE DETERMINATION OF CURD TENSION BY THE USE OF HYDROCHLORIC ACID–PEPSIN COAGULANT[1]

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INTRODUCTION

Within recent years considerable emphasis has been placed upon the curd character of milk in relation to infant feeding. The measurement of the toughness of the curd has become a useful criterion of the suitability of different milks. Buckley (2) in 1914 used dilute hydrochloric acid as a flocculating agent, and observed a marked variation in the curd character of various milks. In this manner he was able to differentiate the milks of various species of lactating animals. Alleman and Schmid (1) in 1916 devised a "tension-knife" of concentric rings to measure the toughness of milk coagula. These investigators coagulated 400 cc. of milk with 40 cc. of water at 35° C. by using 4 cc. of rennet extract. Milk having a short coagulation time was found to have a firmer curd tension. In 1923 Hill (4) described a method for the measurement of the hardness of the curd coagulated by pepsin in calcium chloride solution. He used a knife having radial blades joined to an upright slender handle. This knife is placed in a jar containing 100 cc. of milk brought to 35° C. Then 10 cc. of a coagulant consisting of three parts of a 0.6 per cent solution of 1 to 3,000 scale pepsin to one part of 37.8 per cent calcium chloride is added with slight stirring. After a ten minute interval the loop of the knife is hooked to a spring balance which registers in grams the pull necessary to cut through the curd.

Various workers (5, 8, 10) have demonstrated that coagulation is very slow in calcium-deficient milks. Calcium chloride additions, in large quantities, to milk may retard rennin coagulation, while Rona and Gabbe (9) have observed that smaller quantities may hasten the reaction.

For a period of nearly two years curd tensions were determined monthly by Hill's method on herd samples of three types of milks, which were available, namely the milk of Holstein and Jersey cows and mixed milk of Sanaan and Toggenburg goats at the United States Animal Hus-

Received for publication November 14, 1934.

[1] The modification of the Hill Method for curd tension herein presented was the outgrowth of studies on goats' milk, the results of which are in progress of publication. The author is indebted to J. A. Gamble and A. K. Besley for their cooperation in making possible the study of curd tension measurement, to Paul E. Howe for encouragement and advice during the progress of the work and to N. R. Ellis under whose supervision the work was conducted.

bandry Experiment Farm at Beltsville, Maryland. Later when the milks were treated in various ways such as boiling, homogenizing, skimming, and calcium chloride addition, it was noticed that the calcium chloride of the coagulant exerted various undesirable influences on the curd tensions of the altered milks. For example, the analyses of the curds produced by the use of a calcium chloride-pepsin coagulant as used by Hill (4) revealed a high content of calcium, due apparently to the absorption of calcium chloride from the coagulant. It was also impossible to precipitate the albumin according to the method of the Association of Official Agricultural Chemists at the usual hydrogen-ion concentration. Hence, it seemed that if the ionic equilibrium really plays a rôle in the coagulation mechanism of milk then the calcium chloride in the coagulant must exert some undue influence. Furthermore, if the curd tension is to be a measure of gastric coagulation, then the coagulant used *in vitro* should simulate the gastric juice *in vivo,* in respect to the hydrochloric acid concentration. There appears to be no physiological reason for the presence of calcium chloride in the coagulant, and certainly not in the quantity used, for it has been shown (6, 7) that the calcium content in human gastric juice averages between 0.001 and 0.01 per cent. Therefore, the purpose of the studies described in this paper was to determine the suitability of hydrochloric acid in place of the calcium chloride in the coagulating medium.

EXPERIMENTAL PROCEDURE

The hydrochloric acid concentration in gastric juice (3) is held to vary from 0.2 per cent to 0.5 per cent. Therefore, a study was made of the effect of coagulants containing hydrochloric acid. Solutions were prepared by dissolving 0.45 gram of pepsin in 100 cc. of hydrochloric acid of the various concentrations of 0.2 to 0.5 per cent, inclusive. The technique used was the same as that described by Hill, including the 10 cc. amount of the coagulant. The curd tension values found were practically alike when the hydrochloric acid concentrations varied from 0.2 to 0.5 per cent. Evidently, variations of these concentrations of hydrochloric acid exert only slight changes. It was therefore decided to use 0.4 per cent acid concentration because it approximates the optimum hydrogen-ion concentration for the pepsin.

All samples of milk were studied in duplicate and when possible in triplicate at the same time comparatively using the calcium chloride pepsin coagulant described by Hill and the hydrochloric acid-pepsin coagulant outlined above.

RESULTS

As is shown in Table 1, the curd tensions of all three milks in the raw state obtained by the calcium chloride method (calcium chloride-pepsin

TABLE 1

Comparison of curd tension readings of raw and treated milk as determined with the calcium chloride and hydrochloric acid coagulants and the percentage changes in the curd tensions of the treated milks compared with the corresponding raw milk curd tensions

TREATMENT OF MILKS	NO. OF SAMPLES	Coagulant Used			
		$CaCl_2$–pepsin		HCl–pepsin	
		In grams	In per cent change*	In grams	In per cent change*
GOAT MILK					
Raw	6	27	.....	37	.....
Boiled	6	15	−45	14	−62
Homogenized	2	21	−22	21	−43
Skimmed	5	34	+26	43	+16
Skimmed and boiled	5	17	−37	13	−65
Calcium chloride added	12	30	+11	31	−16
HOLSTEIN MILK					
Raw	5	51	.....	53	.....
Boiled	5	32	−37	15	−72
Homogenized	2	35	−31	29	−45
Skimmed	5	55	+ 8	59	+11
Skimmed and boiled	2	29	−43	9	−83
Calcium chloride added	2	46	−11	46	−13
JERSEY MILK					
Raw	6	68	.....	75	.....
Boiled	6	54	−21	32	−57
Homogenized	2	64	− 6	56	−25
Skimmed	5	85	+25	89	+19
Skimmed and boiled	2	66	− 3	36	−52
Calcium chloride added	2	70	+ 3	90	+20

* Negative sign prefixed to the per cent change indicates decrease in curd tension or softening of the milk curd.

Positive sign indicates increase in curd tension or hardening of the milk curd.

coagulant) were always lower than those yielded by the acid method (hydrochloric acid-pepsin coagulant). By no means were the acid curds uniformly higher for the three milks. The modified coagulant produced curd tensions of goat milk 37 per cent higher than when the calcium chloride coagulant was used, Holstein milk 4 per cent higher, and Jersey milk 10 per cent higher.

When samples of the milks were boiled for one minute, the average curd tension by the calcium chloride method of the goat milk dropped from 27 to 15, the Holstein milk from 51 to 32, and the Jersey milk from 68 to 54. However, the acid coagulant, though yielding higher curd tensions for the raw milks, showed greater softening due to boiling of the three types of milk. The acid coagulant revealed a more marked softening of

the boiled Holstein and Jersey milks than was demonstrated by the calcium chloride coagulant. For example, the raw goat milk showed a curd tension of 37 by the modified coagulant and when boiled 14, that of Holstein milk dropped from 53 to 15, and that of the Jersey milk from 75 to 32. In terms of percentage, the degree of softening as shown by the calcium chloride coagulant is 45 per cent for goats' milk, 37 per cent for Holstein, and 21 per cent for Jersey milks, while the curd tensions of the same milks by the acid coagulant were softened 62 per cent, 72 per cent and 57 per cent, respectively.

Results on the three types of milk, when homogenized unpasteurized at a temperature of 48° C. at 4,000 pounds' pressure and then tested for the extent of the softening by the two coagulants, showed a greater drop in hardness of the curd of the raw milk due to homogenization when tested by the acid coagulant than was shown by the calcium chloride coagulant. Goat milk, when homogenized and tested by the calcium chloride coagulant, showed a decrease in its curd tension of 22 per cent, Holstein milk, 31 per cent decrease, and Jersey, 6 per cent. Yet the same milk by the acid coagulant revealed greater softening, namely, 43 per cent, 45 per cent, and 25 per cent, respectively.

All milks when skimmed had tougher curds than the corresponding whole milks. The skimmed goat, Holstein and Jersey milks gave curd tensions of 34, 55 and 85, respectively, by the calcium chloride method, while the acid method revealed higher readings for all three milks. The curd tension of the goat skimmed milk tested by the calcium chloride method increased 26 per cent, the Holstein milk, 8 per cent, and the Jersey milk, 25 per cent over the respective whole milks. On the other hand, the same milks showed increased toughness of 16, 11, and 19 per cent, respectively, when tested by the acid method.

Both coagulants indicate that the boiled skimmed milks were softened to the same extent as the boiled whole milks, but the acid coagulant apparently revealed a greater softening of the Holstein and Jersey milks than the calcium chloride coagulant. The tendencies of the acid coagulant seem to be consistent throughout. As compared to the calcium chloride coagulant, the acid coagulant resulted either in the greater hardening of the curd or in greater softening, depending on the treatment of the milk. It is possible that this reaction of the acid coagulant indicates greater sensitivity to changes in the milk.

The addition of calcium chloride to milk previous to coagulating by the two coagulants was studied for the purpose of determining the influence of calcium chloride on the curd tension. To each 100 cc. of milk, 0.5 gram of calcium chloride in the form of a 20 per cent solution was added and stirred before the addition of the coagulant. In case of the goat and

Holstein milks, the two coagulants produced identical curd tensions for the corresponding milk, indicating that the calcium chloride depressed the coagulation by the acid method to the same curd tension as the calcium chloride method, but in the case of the Jersey milk, the modified coagulant was influenced in the opposite direction, resulting in a higher curd tension.

DISCUSSION

The fact that the coagulant containing calcium chloride with pepsin as suggested by Hill (4) yielded lower results for the raw milks than the modified coagulant with hydrochloric acid replacing the calcium chloride evidently indicates the retarding effect due to the addition of more calcium chloride in the former coagulant. From the results obtained, it would seem that the depressant effect of the calcium chloride coagulant is not uniform because of the variable salt composition of each milk.

Evidently, heat increases the coagulation time, hence the softer curd, but the addition of calcium chloride almost causes a return to normal coagulation due apparently to the replacing of the precipitated calcium. The two coagulants show their greater disparity in the curd tensions of boiled milks where the acid coagulant produced softer curds than did the calcium chloride coagulant and yet the former showed that greater softening occurred in the boiled milks. Furthermore, the boiling of milk is known to produce a soft curd. For that reason one would question whether a drop in Jersey milk from 68 to 54 when boiled and tested by the calcium chloride coagulant can be considered marked softening when soft curds are classified by Hill to be below 30. This finding is more dubious because when tested by the modified coagulant the boiled Jersey milk is softened from 75 down to 32.

As previously mentioned, skimmed milks were consistently tougher than their corresponding whole milks. Data from an unpublished manuscript on curd tension of goat milk show statistically that the fat in the milk plays only a slight rôle in the curd solidity. The fat in milk tends mechanically to prevent the formation of a solid mass due to the interspersed enmeshment of the fat globules among the coagulated protein particles. Otherwise, the correlation factor is insignificant for the fat content. But a highly significant correlation exists between protein content and the curd tension.

Again, even in the case of skimmed milks, the acid coagulant revealed that boiling caused a greater softening of all milks, including the skimmed, than the calcium chloride coagulant. Such data seem to indicate that the calcium chloride coagulant can not be used for the determination of the curd tension of altered milks if results are to be obtained comparable to those which will approximate the coagulation within the stomach. It is

therefore felt that the acid coagulant is a truer *in vitro* representation of gastric coagulation, especially since the gastric juice contains relatively little calcium, that is, less than 0.01 per cent.

Obviously, the fact that in raw milk the curd tensions were lower when tested by the calcium chloride coagulant than when tested by the modified acid coagulant would seem to indicate the depressing effect of calcium chloride. Most likely this retarding effect of calcium chloride already present in the calcium chloride coagulant is at its maximum as is evidently shown by the results obtained when 0.5 gram more of calcium chloride is added to the milk before coagulation. The additional calcium chloride exerts no further influence on the curd tensions of the three milks by the calcium chloride coagulant. On the other hand, the acid coagulant reveals that milk supplemented with 0.5 gram of calcium chloride before adding this acid coagulant is decreased to that given by the calcium chloride before adding this acid coagulant. Obviously, the calcium chloride of the calcium chloride coagulant exerts an undesirable influence if one wishes to study the effects of various additions to milk.

SUMMARY

1. Excess calcium chloride as is present in the coagulant suggested by Hill which contains calcium chloride and pepsin retards coagulation and hence produces a subnormal curd tension.

2. Evidence indicates that a coagulant of 0.45 gram of pepsin per 100 cc. of 0.4 per cent hydrochloric acid produces a truer picture of the curd character simulating the gastric conditions than one which contains calcium chloride.

3. Boiling for one minute softens the curd of all milks, but not to the same degree of softness. The curd tension of the calcium chloride coagulant of boiled Jersey milk revealed only a slight softening, but did show a marked drop by the acid coagulant.

4. The effects of the various treatments of the milks in terms of percentage increase or decrease of the curd tensions were almost alike for the three kinds of raw whole milks when tested by the acid coagulant.

5. On the other hand, the calcium chloride coagulant on the same milks produced effects of a more divergent percentage increase or decrease in curd tension readings.

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RELATION OF THE PROTEOLYTIC ENZYME ACTIVITY TO THE PROTEOLYTIC ORGANISMS FOUND IN SEPARATOR SLIME

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Several types of microorganisms are of importance in causing the deterioration of cream used for butter making, and the action of the proteolytic organisms, which correspond in a great measure to the tryptic and ereptic enzymes of animal origin and in some instances the action of pepsin-like enzymes, is manifested in the course of milk and cream hydrolysis.

In the following experiments the object has been to study the relation of the activity of the proteolytic enzymes in separator slime to the numbers of proteolytic organisms present. Separator slime, rather than the milk or cream, was used because the slime represents a composite sample of a large quantity of milk or cream. The slime was obtained from about 300 gallons of milk delivered by producers to a commercial milk plant. This milk was bought on grade basis and represented a high grade of market milk.

METHODS OF PROCEDURE FOR THE STUDY OF THE ENZYMES

The separated slime was triturated in a mortar with a 0.7 per cent salt solution until the slime was thoroughtly disintegrated, then rubbed through a coarse sieve. The salt solution represented half of the mixture.

Of this mixture 10 cc. were added to 200 cc. of sterile milk and digested at 37° C. for five days with sufficient toluol to completely saturate the mixture leaving a slight excess on the surface. An analysis of the mixture was made before digestion for soluble nitrogen compounds which were not precipitated by phosphotungstic acid and by a saturated solution of zinc sulphate, these results constituted the base from which the increases were determined. After five days digestion an analysis was again made. The difference between the last analysis and the first represented the increase of soluble nitrogen compounds not precipitated by phosphotungstic acid and by zinc sulphate.

Three samples of slime were taken during the first week of each month throughout the experiment of fourteen months. The separate analyses of the three periods were averaged and the result taken to represent the changes for the specified month. This procedure gave a better average representation of the bacterial count and enzymatic activity.

The total bacterial count was secured by plating known quantities of slime in duplicate upon beef infusion agar prepared from dehydrated

Received for publication December 3, 1934.

Bacto beef; counts were made after five days incubation at 21° C. The proteolytic count was secured by plating known quantities of slime upon agar containing 10 per cent milk and incubation was for five days at 21° C. Colonies which showed definite clearing were counted as proteolytic, doubtful colonies were transferred into diluted litmus milk diluted 50 per cent with distilled water, and proteolysis was recorded if present after ten days incubation at 21° C.

Table 1 gives the results of the analysis for each month. The total and proteolytic bacterial counts are expressed as the logarithm of the number in one gram of slim mixture. The nitrogen is expressed as percentage of the total nitrogen.

TABLE 1

Proteolytic enzymatic activity of separator slime as influenced by the month of the year and its bacterial content

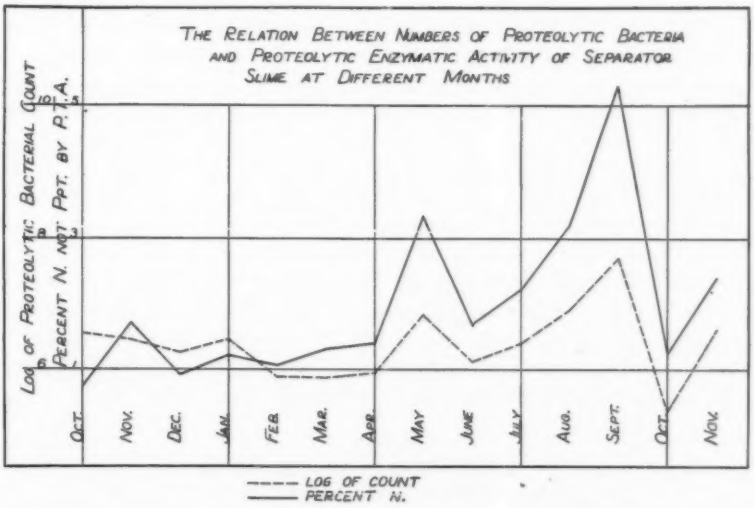
	LOGARITHM OF BACTERIAL COUNT:		PER CENT NITROGEN NOT PRECIPITATED BY:		
Date	Total bacteria	Proteolytic bacteria	Phospho-tungstic acid	Zinc sulphate	Peptones
October	7.95	6.56	0.75	1.16	0.41
November	8.00	6.45	1.71	1.78	0.07
December	7.56	6.26	0.85	1.49	0.64
January	7.78	6.46	1.22	2.23	1.01
February	7.18	5.90	1.06	1.14	0.08
March	7.26	5.87	1.31	1.53	0.22
April	7.52	5.95	1.40	1.61	0.21
May	8.23	6.83	3.36	5.00	1.65
June	9.00	6.11	1.68	4.55	2.87
July	7.66	6.42	2.24	3.93	1.69
August	7.82	6.93	3.20	5.04	1.84
September	8.59	7.70	5.31	6.80	1.49
October	7.04	5.38	1.25	1.44	0.19
November	7.89	6.61	2.40	2.76	0.36

In the study of Table 1 it is well to consider the relation of the total bacterial count and the count of the proteolytic types of bacteria. The average total count of bacteria for the winter months, that is: October, November, December, January, February, March and April, was 37.59 millions or a logarithm of 7.57. For the five summer months, May, June, July, August and September, the total average bacterial count was 186 millions or a logarithm of 8.26. This shows that the slime produced in summer months contained 148.4 millions more bacteria than the slime produced in winter months. By comparing the average count for the winter months with the average count for the summer months we find the number of bacteria for the summer months was 4.94 times that of the winter months.

If we consider the proteolytic types of bacteria a similar relation exists for the summer and winter months, the total average count of the proteolytic types for the winter months was 1.63 million corresponding to a logarithm of 6.21 while for the summer months the total average was 6.31 millions corresponding to a logarithm of 6.80. The summer months contained 4.68 million more bacteria than the winter months or 3.87 times more for summer than for the winter months.

Of the total bacterial count only approximately 5 per cent appeared to be of the proteolytic type. This is true for the summer months as well as for the winter months.

The graph shows the relation between the average proteolytic count for the month and the average percentage of nitrogen not precipitated by phosphotungstic acid for the month.



In discussing the proteolytic action of the bacterial enzymes on the proteins of milk we are taking into consideration only the proteolytic types of bacteria. The hydrolized products produced by the proteolytic enzymes of these organisms are expressed as nitrogen compounds not precipitated by phosphotungstic acid and nitrogen compounds not precipitated by saturated solution of zinc sulphate. The relation of these two fractions gives an index as to the character of the enzymes produced by the respective organisms. The statement formerly made by us, (1), (2) corroborated by H. von Euler (34), W. Grassman (4), and Franz Fuhrman (5), that most bacterial enzymes are of the tryptic and ereptic types. The tryptic type hydrolizes the proteins to the peptone and dipeptid state, and the ereptic

type hydrolizes the dipeptids into animo acids. The animo acids formed appear in the fraction not precipitated by phosphotungstic acid, the dipeptids, and polypeptids (peptones) together with the amino acids appear in the fraction not precipitated by zinc sulphate, therefore, the difference of the nitrogen compounds not precipitated by zinc sulphate and phosphotungstic acid represent the peptones. This gives at least two well defined fractions of nitrogen compounds. In addition to the animo acids not precipitated by phosphotungstic acid there are some soluble compounds of nitrogen not amino acids; these same soluble compounds also appear in the nitrogen compounds not precipitated by zinc sulphate.

If we compare the different soluble compounds produced by the bacterial enzymes on milk proteins during the winter months with that of summer months we find that the ratio is 1.0 to 2.12 for nitrogen not precipitated by phosphotungstic acid, and the ratio for nitrogen compounds not precipitated by zinc sulphate is 1.0 to 2.87, which means that the hydrolysis of enzymatic activity is 2 to 3 times greater for the summer months than for the winter months. Referring this change to the proteolytic bacterial count there is shown a good relation, but not proportional to the increase in bacterial count for the winter and summer months. The bacterial ratio for winter and summer months is 1.0 to 2.9. The increase of the proteolytic bacteria increased 3.87 times during the summer months while the protein hydrolysis increased approximately three times, as summarized in activity, this relative activity of bacterial enzymes is well illustrated in Table 2. The proteolytic enzymes of proteolytic bacteria vary in their our previous work (2). A comparison of the proteolytic bacteria *B. ichthyosmius, A. putrefaciens,* and *S. liquefaciens* illustrate the possibility that the number of proteolytic bacteria do not of necessity imply a corresponding degree of enzyme activity on the proteins of milk.

TABLE 2

Ratio increase of bacteria and proteolytic enzymes in separator slime obtained during winter months to that of summer months

TOTAL	BACTERIA PROTEO.	N NOT PPT BY P.T.A.	N NOT PPT BY $ZnSO_4$	PEPTONE
4.94	3.87	2.12	2.87	5.45

THE INACTIVATION OF THE PROTEOLYTIC ENZYMES OF SEPARATOR SLIME BY HEAT

It was deemed advisable to study the effect of heat on the activity of the proteolytic enzymes as found in separator slime. After the addition of the separator slime to the milk the mixtures were heated to temperatures

of 145° F. for 30 minutes and at 165° F. for 10 minutes, the mixtures cooled, toluol added and the mixture digested for 5 days at 37° C. Prior to pasteurization the milk was adjusted to two pH levels, 7.0 and 5.5. The per cent inactivation of the proteolytic enzymes as found in two samples of separator slime with widely different proteolytic bacteria contents is given in Table 3.

TABLE 3

The effect of heat on enzymatic inactivation of separator slime

	HEATED TO 145° F. FOR 30 MINUTES PER CENT INACTIVATED AT				HEATED TO 165° F. FOR 10 MINUTES PER CENT IN ACTIVATED AT			
	pH 7		pH 5.5		pH 7		pH 5.5	
	N not ppt by P.T.A.	N not ppt by $ZnSO_4$	N not ppt by P.T.A.	N not ppt by $ZnSO_4$	N not ppt by P.T.A.	N not ppt by $ZnSO_4$	N not ppt by P.T.A.	N not ppt by $ZnSO_4$
Sample I	73	78	77	60	72	71	74	72
Sample II	67	47	70	64	66	69	61	67

Sample I had a protelytic bacterial count of 40 million per cc. while Sample II had a proteolytic bacterial count of 2 million or in terms of logarithms respectively 7.6 and 6.3.

In this experiment the effect of pH 7 and pH 5.5 was also studied, the results of which are given in table 4.

TABLE 4

The effect of acidity of substrate upon the enzymes found in separator slime

	AT pH 7		AT pH 5.5		LOGARITHM OF PROTEOLYTIC BACTERIA COUNT
	N not ppt by P.T.A.	N not ppt by $ZnSO_4$	N not ppt by P.T.A.	N not ppt by $ZnSO_4$	
Sample I	3.23	4.59	1.57	2.88	7.6
Sample II	1.89	2.46	1.15	3.19	6.3

As to the relation of the bacterial count to the enzymatic activity we find that the proteolytic activity is much greater for Sample I than for Sample II, the latter containing a relatively smaller number of bacteria. The effect of pH on bacterial enzyme action is consistent with our former work on the proteolytic action of the enzymes of specific organisms, that is, the activity, in general, is greater at pH 7 than at pH 5.5.

The inactivation by heat, whether for 145° F. for 30 minutes or at 165° F. for 10 minutes is approximately the same, which agrees with our previous work on the enzymes of *B. ichthyosmius* and *A. putrefaciens*. The killing effect of heat depends on two factors, temperature and time. The inactivation was slightly greater for pH 7 than for pH 5.5; however,

the difference is not significant. It will also be noted that the percentage of inactivation was greater for the enzymes in Sample I than in Sample II, probably due to the different types of bacteria the enzymes of which are less sensitive to heat. Approximately 30 per cent of the activity remained after heating the separator slime to the temperature and time, as used in the experiment. This corroborates the statements frequently made that pasteurization does not destroy all the enzymatic action which contributes to the deterioration of dairy products.

SUMMARY

1. The proteolytic enzymatic activity of separator slime was greater during the summer months than during the winter months.

2. There exists a relation between the numbers of proteolytic bacteria in separator slime and its proteolytic enzymatic activity.

3. Approximately 70 per cent of the proteolytic enzymatic activity of separator slime was inactivated at 145° F. for 30 minutes and 165° F. for 10 minutes.

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American Dairy Science Association Announcements

GENERAL INFORMATION

It is possible to announce at this time the general program for the American Dairy Science Association Meeting to be held the week of June 24 at the University of Minnesota. The general committee believes that an especially attractive program has been arranged. The University of Minnesota is preparing to entertain a large percentage of the membership and their families. A full program of special entertainment features is being planned for the ladies which is guaranteed to prevent ennui. This will be announced in the May issue of the Journal.

The members are reminded that titles of papers to be presented must be in the hands of L. S. Palmer, Chairman of the Program Committee by May 1st. Abstracts must be sent with titles or very shortly thereafter if reprints of the abstracts are to be available for the members at the time of the meeting.

L. S. Palmer, *Chairman*
Program Committee

GENERAL PROGRAM

American Dairy Science Association
Annual Meeting, June 24–27, 1935
University Farm, St. Paul

June 24—Monday

9 A. M.–5 P. M.	General registration and room registration, Haecker Hall.
5 P. M.–9 P. M.	General Registration and room registration, Women's Dormitory, University Farm.
2 P. M.	Dairy Products Judging Committee (tentative) Creamery Rooms—Haecker Hall.
7:30 P. M.	Opening Session and Business Meeting, Auditorium, Administration Building, University Farm.
8:30 P. M.	Social get-together for members and their families, Women's Dormitory Parlors, University Farm.

June 25—Tuesday

8 A. M.–4 P. M.	General registration and room registration, Haecker Hall.
8 A. M.–9 A. M.	Section Committee Meetings (Rooms will be announced at opening meeting Monday evening).
9 A. M.– 12 noon	Joint Symposium "Improving the Germ Plasm of Domestic Plants and Animals," Auditorium, Administration Bldg., University Farm.

This symposium will be under the auspices of the following organizations:
Section O, American Association for the Advancement of Science
The American Dairy Science Association
The American Society of Agronomy (Corn Belt Section)
The American Horticultural Society (Great Plains Section)
The American Society of Plant Physiologists
The American Phytopathological Society

JUNE 25—TUESDAY (continued)

12 noon–1 P. M.	Lunch hour.
1 P. M.–4 P. M.	Section scientific meetings.
4 P. M.–4:30 P. M.	Section Committee meetings.
4 P. M.–4:30 P. M.	Members and their families leave for trip to Land-O-Lakes.
6 P. M.	Complimentary dinner and entertainment at Land-O-Lakes. Admission by ticket.

JUNE 26—WEDNESDAY

9 A. M.–12 noon	Section Scientific meetings.
12 noon–1 P. M.	Complimentary Dairy lunch.
1 P. M.–2 P. M.	Section business meetings.
2 P. M.–4:30 P. M.	Section scientific meetings.
5 P. M.	Leave for Minneapolis Automobile Club.
7 P.M.	Subscription banquet and entertainment at Minneapolis Automobile Club. (Tickets to be purchased on registration.)

JUNE 27—THURSDAY

9 A. M.–10 A. M.	Closing general session and business meeting, Auditorium, Administration Building, University Farm.
10 A. M.–1 P. M.	Section scientific meetings.
2 P. M.	Leave for arranged tours to cooperative and independent dairy plants in the Twin Cities and trips to farms of various breeders in the vicinity of the Twin Cities. Information regarding a number of these suggested tours and trips will be given members as they register, and the request will be made to signify definitely at that time which, if any, of these tours or trips is desired. This information will be required in order that necessary transportation can be arranged and the organizations or farms to be visited may be notified of the number of visitors to be expected.

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